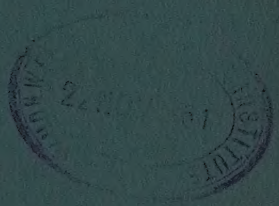
PAPERS OF THE MICHIGAN ACADEMY OF SCIENCE ARTS AND LETTERS

XXXV (1949)

PART I: BOTANY AND FORESTRY



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VOLUME XXXV IS AVAILABLE IN FOUR PARTS:

PART I: BOTANY AND FORESTRY

PART II: ZOOLOGY

PART III: GEOGRAPHY AND GEOLOGY

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Anthropology, Language and Literature, Medical Science, Philosophy, Political Science, Psychology

PAPERS OF THE MICHIGAN ACADEMY OF SCIENCE ARTS AND LETTERS

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FREDERICK K. SPARROW

VOLUME XXXV (1949)

PART I: BOTANY AND FORESTRY

"Pusilla res mundus est nisi in illo
quod quaerat omnis mundus habeat."
— SENECA, *Naturales Quaestiones*

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BOTANY

A SEARCH FOR THE CORRECT COLCHICINE TREATMENT OF THE EMBRYOS OF *SCILLA SPP.* TO PRODUCE POLYPLOIDS*

WILLEM EDUARD DE MOL

INTRODUCTION

IN EXPERIMENTS with Fragaria, Nicotiana, Petunia, and Rubus, Derman (1938) proved the value of colchicine treatment of seed plants in which embryonic development takes place close to the surface of the ovary. Two years later De Mol (1940) described colchicine experiments with Bellevallia. The present paper deals with experimental methods adapted to various bulbous and tuberous plants to obtain clones with double the usual numbers of chromosomes.

Varieties of *Scilla sibirica* and *Scilla tubergeniana* were used in the experiments. These Scillae were chosen from among the bulbous plants because their exceptionally flexible flower stalks (especially *S. sibirica*) are well adapted to the method of treatment. Also, a bulb bears several stalks, each with a number of flowers. This habit provides a great many embryos per bulb. Although hyacinths have a large number of flowers, the stiffness of the stem and the arrangement of the flowers cause mechanical disadvantages in treatment. Tulips and daffodils mostly produce single flowers.

Four bulbs of *S. sibirica,* four of the triploid variety Spring Beauty (De Mol, 1942), and three of *S. tubergeniana* were potted at the end of October, 1945. Flowering started in an unheated greenhouse on the south side of the building on February 1. On February 10, after removal of the stamens, the first cross-pollinations were performed. Only the earliest flowers of two or three flower stalks were used; later ones were removed. Colchicine was applied 4, 9, 23, and 39 days after pollination.

* *Acknowledgment:* The author is indebted to the University of Amsterdam for laboratory facilities and to Dr. Teunis Vergeer, of Hope College, Holland, Michigan, for valuable assistance in the preparation of the manuscript.

Two methods of treatment were employed:

(1) A drop of the colchicine solution was placed on the ovary after the anthers had been completely removed. The surrounding petals were relied on to hold the solution in contact with the ovary. This method is satisfactory only while the petals remain turgid.

(2) The flower stalk having only the ovaries is bent into a small tube containing the colchicine solution and is held in place with a plug of cotton. This method prevents evaporation, and therefore little of the solution is lost. As controls, a number of flowers without stamens were allowed to develop naturally. Since experiments by other workers have proved that 2 per cent colchicine is effective, this amount was used in these experiments.

TABLE I

S. TUBERGENIANA ♀ × S. SIBIRICA ♂

Date of pollination	Date of treatment	Days between pollination and treatment	Treatment in hours	Method	No. of flowers	Ovary March 28	Ovary April 10	Yield May 15
Feb. 10	Feb. 14	4	6.5	Drop applied	1	Some swelling	Died off	None
Feb. 10	Feb. 16	6	24	Drop applied	1	Became yellow	Died off	None
Feb. 10	Feb. 18	8	9	Tube	1	Became yellow	Died off	None
Feb. 11	Feb. 15	4	48	Drop applied	1	Withered		None
Feb. 11	Feb. 17	6	7	Tube	2	Some swelling	Died off	None
Feb. 17	Feb. 23	6	6	Tube	3	No swelling	Died off	None
Feb. 18	Control	..	..	..	5	Normal swelling	Four died off	Three seeds

TABLE II

S. TUBERGENIANA ♀ × ♂ (SELF-POLLINATED)

Date of pollination	Date of treatment	Days between pollination and treatment	Treatment in hours	Method	No. of flowers	Ovary March 28	Ovary April 10	Yield May 15
Feb. 10	Feb. 14	4	4	Drop applied	1	Some swelling	Died off	None
Feb. 10	Feb. 16	6	6	Drop applied	1	Some swelling	Died off	None
Feb. 10	Feb. 18	8	8	Tube	2	Some swelling	Died off	One seed
Feb. 13	Feb. 19	6	6	Tube	1	Became yellow	Died off	None
Feb. 15	Feb. 19	39	14	Tube	3	Normal swelling	Normal swelling	Three seeds
Feb. 10	Control	..	..	..	1	Some swelling	Died off	None

The results of pilot experiments to determine the most favorable time lapse between pollination and treatment, the best length of exposure, and the most favorable crosses are given in Tables I–VI.

Tables I and II show that only very few seeds came to full development. This was true, however, of the controls and of the open-ground seed yield of these species. For example, in 1945 only 26 seeds were harvested from 16 deliverable bulbs. For the remaining experiments a species or a variety was used which, under normal circumstances, yields seeds abundantly. The temperature at which the crossings were performed was 3°–4° C., which was low.

The forming of the embryo under the influence of the solution probably took place only in the case of treatment after 8 days for 8 hours. Further breeding will determine this. The treatment for 14 hours 39 days after pollination is of very questionable value. Because of their low fertility these crosses were not repeated.

TABLE III

S. SIBIRICA ♀ × S. TUBERGENIANA ♂

Date of pollination	Date of treatment	Days between pollination and treatment	Treatment in hours	Method	No. of flowers	Ovary March 28	Ovary April 10	Yield May 15
Feb. 29	March 8	8	1	Tube	3	Normal swelling	Normal swelling	Seven seeds

TABLE IV

S. SIBIRICA ♀ × S. SIBIRICA VAR. SPRING BEAUTY ♂

Date of pollination	Date of treatment	Days between pollination and treatment	Treatment in hours	Method	No. of flowers	Ovary March 28	Ovary April 10	Yield May 15
Feb. 23	March 1	7	5	Tube	2	Normal swelling	Normal swelling	Seven seeds
March 5	March 9	4	4	Tube	3	Normal swelling	Normal swelling	One seed
Feb. 23	Control	..	..	..	3	Normal swelling	Normal swelling	Eight seeds

Since Tables III and IV show that *S. sibirica* sets seed more easily than *S. tubergeniana*, the experiments with it will be continued on a larger scale.

A period of four days between pollination and treatment is not too short for embryo formation. In the experiment, however, only one seed

resulted from three flowers. When seven days were allowed to elapse between pollination and a treatment, seven seeds developed from two flowers.

It would appear, then, that there are about five days between pollination and embryo formation in *S. sibirica*. New experiments are needed to answer this question.

It is interesting to note that pollen of Spring Beauty is fertile enough to produce considerable seed in spite of the fact that it is triploid (De Mol, 1942).

Table V shows that a cross between Spring Beauty and *S. tubergeniana* as the male parent is also sterile, even when ample time is allowed for the formation of the embryo (23 days).

TABLE V

S. SIBIRICA VAR. SPRING BEAUTY ♀ × S. TUBERGENIANA ♂

Date of pollination	Date of treatment	Days between pollination and treatment	Treatment in hours	Method	No. of flowers	Ovary March 28	Ovary April 10	Yield May 15
Feb. 22	Feb. 29	7	2	Tube	2	Some swelling	Died off	None
March 2	March 25	23	5	Tube	2	Some swelling	Died off	None

TABLE VI

S. SIBIRICA VAR. SPRING BEAUTY ♀ × S. SIBIRICA ♂

Date of pollination	Date of treatment	Days between pollination and treatment	Treatment in hours	Method	No. of flowers	Ovary March 28	Ovary April 10	Yield May 15
Feb. 22	March 2	9	2	Tube	1	Some swelling	Died off	None
March 8	March 12	4	1.5	Tube	2	Normal swelling	Died off	Three seeds
March 23	March 30	7	6	Tube	2	Normal swelling	Died off	Two seeds
March 28	April 4	7	6	Tube	2	Normal swelling	Died off	Three seeds
March 8	Control	..	..	..	2	Normal swelling	Died off	Eight seeds

Table VI shows that, in spite of colchicine treatment, seeds form fairly readily from *S. sibirica* var. Spring Beauty when crossed with the original species.

Treatment of 1.5 hours does not markedly interfere with embryonic development when only 4 days are allowed to pass between pollination

and treatment. Potentially, normal *S. sibirica* can produce 20 embryos from an ovary. Since few seeds were obtained, it is believed that they developed in spite of the colchicine treatment. They cannot at this time be sacrificed for microscopic examination, and only culture can determine whether polyploid embryos resulted from the treatment.

SUMMARY

Of the bulbous plants, *Scilla sibirica* was found to be best adapted for treatment by colchicine. Crosses of *S. tubergeniana* ♀ with *S. sibirica* ♂ proved almost sterile, as was the self-fertilized *S. tubergeniana*. Crosses of *S. sibirica* ♀ with *S. tubergeniana* ♂ were more productive. Crosses of *S. sibirica* ♀ by *S. sibirica* var. Spring Beauty ♂ yielded good seed, as did reciprocal crosses.

From the study of several crosses it appears that four days must elapse between pollination and treatment if any seed is to develop, but more seed results if an interval of seven days is allowed. Partial inhibition of seed formation is interpreted as an indication of the effect of colchicine.

The duration of exposure to 2 per cent colchicine extended from one to forty-eight hours. Treatment as short as one and one-half hours reduced the normal seed yield.

Amsterdam, The Netherlands

LITERATURE CITED

De Mol, W. E. 1940. Morphologische und cytologische Abweichungen bei Bellevallia usw. durch Colchicin, sowie der theoretische und practische Wert der Colchicin-Behandlung im Vergleich zu dem Werte anderer Mittel. La Cellule, 48 : 259–276.

—— 1942. Die erste gezüchtete Triploide *Scilla sibirica* Andrews. Die Gartenbauwissenschaft, 17, Heft 3 : 227–239.

Derman, H. 1938. A Cytological Analysis of Polyploidy Induced by Colchicine and by Extremes of Temperature. Journ. Hered., 29 : 210–229.

TWENTY-FIVE YEARS OF TULIP IMPROVEMENT BY X-RAY*

WILLEM EDUARD DE MOL

INTRODUCTION

THE history of the European culture of tulips dates back nearly four centuries, when the first were imported from Turkey. In 1581 Matthias de Lobel described twenty-two varieties in his *Kruidtboeck* ("Book of Herbs"). The first mention of sowing tulip seed was made by Crispyn van de Pas in 1636. In the seventeenth century Dutch "tulipmania" reached the point at which 11,500 guilders, or about $4,600, was paid for a single bulb of a new variety. Double tulips were first recorded in 1665. Parrot tulips were known before 1800. The Darwin tulips that are popular at present were largely originated by hybridization; others arose by mutations and were recognized as such during the early part of this century, when the author first undertook the study of bulb culture. Early experiments with X-ray treatment of tulips produced the mutation Sparkling Pitt from the tulip William Pitt (Pl. I). Because it combined several improvements in one flower (see below) it markedly stimulated interest in this manner of producing mutations.

SPONTANEOUS MUTATIONS

Mutations in flower bulbs are no surprise to the grower or the experimenter. During a culture of many years spontaneous changes have been observed under field conditions. These mutated plants were isolated and multiplied vegetatively; they have preserved the mutated quality. Most of them have been propagated in great quantities as precious assets and, together with the mother varieties, have been marketed for many years; for example, Cordell Hull, Bonifacius, Merula, Monarch, Hudson, Hilde-

* *Acknowledgment:* The author is indebted to the laboratories of the University of Amsterdam and to Philips Ltd., Electric Works, Eindhoven, Netherlands, for the use of X-ray equipment and other facilities, and to Dr. Teunis Vergeer, of Hope College, Holland, Michigan, for valuable assistance in the preparation of the manuscript.

brand, Sedan, Tindal, and Unitas have been grown from the red Darwin tulip Bartigon. They represent as many somatic mutations in color from pink to red.

MUTATIONS DUE TO ALTERATION OF TEMPERATURE

That high- or low-temperature treatment during storage results in genetic changes was also established during the first quarter of this century (De Mol, 1929).

X-RAY MUTATIONS

Roentgen rays were first used in 1922. They appear to cause the same mutations as those which originate spontaneously in the field or by modi-

FIG. 1

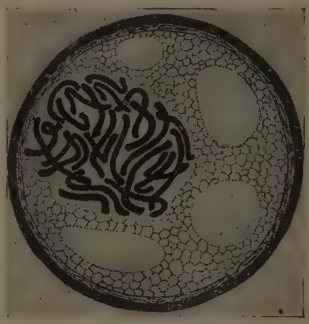

FIG. 2

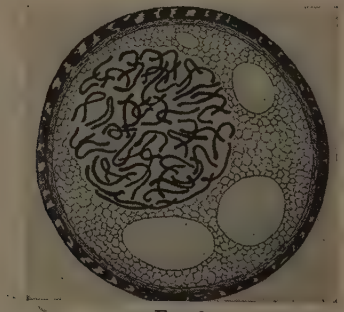

FIG. 3

FIGS. 1–3. Haploid, diploid, and tetraploid pollen of tulips. Pollen grains progressively larger, but all figures are enlarged to same size. Photographs by J. Agterberg

fication of temperature, but they evoke the natural potentialities of tulips much more easily. The treatment is a valuable means of improvement for both the grower and the experimenter, but it cannot in one year produce bulbs with the desired characteristics. The mutation generally first appears as an aberration in color or shape in a single sharply defined sector of a normally colored petal. During the development of the plant in spring new small bulbs grow between the withering scales. They originate from the buds present at the time of planting in October of the preceding year. When these buds are in the mutated sector, the new bulbs retain this feature. One may note from year to year the gradual spread of the mutated characteristic, first to a perianth leaf, then to a sector of the plant, and finally to the whole plant (Pls. II–III).

Between 1928 and 1935 it was discovered that X-raying of hyacinths causes slow and feeble growth and that X-raying of tulips and hyacinths may result in doubling or quadrupling the number of pollen grains (Figs. 1–3). Microscopic studies showed that (1) the number of chromosomes varied owing to the arrest of nuclear division, (2) chromomeres separated from their chromosomes and often were stretched out into filaments, (3) chromosomes remained in the cytoplasm after nuclear reconstruction, (4) many pollen grains became sterile, and (5) somatic mutations also occurred (De Mol, 1933, 1937).

By 1940 the practical application of X-ray treatment had resulted in seventy-two distinct characteristics. The most notable among these represent modifications of the amount of anthocyanin, a blue, violet, or red pigment found dissolved in the cell sap. The variety Dido changed from orange pink to a lighter shade; Prosperity turned pink instead of white; Fantasy changed from pink to red; William Copland, from violet to pink; Roi d'Islande, from pink to violet. Yellow plastids formed in the dark-pink tulip Peach Blossom. Its mutation shows a mixture of red and yellow.

The color of the leaves is often modified; for example, Prince of Austria has a light-green edge, and Fantasy has developed a yellow stripe in the middle of the leaf, whereas green and yellow stripes alternate in Peach Blossom and Gemma (Pl. IV).

The shape of the flowers shows various aberrations after treatment; among them the edge of the perianth leaves is ragged in Van der Neer, the flower has become more globate in Roi d'Islande, the perianth leaves have sharper points in Clara Butt. The Parrot tulips are X-ray bud mutations whose perianth leaves have been enlarged from the normally formed General de Wet (Pl. V). The Parrots Gemma and Fantasy prove

that this process is not irreversible; by continued treatment they have been reduced to the normal flower shape. Fantasy and Red Champion have also developed different shapes and colors (Pl. VI, Fig. 1).

Several mutations may combine in a single new tulip. Sparkling Pitt is a sparkling dark-red bud mutation of the lighter-red William Pitt. The color is finer than that of the mother variety, and the flower, originally rounded off on the under side, has become somewhat squat, and a so-called butterfly heart[1] has developed at the base of the perianth leaves (Pl. I). It is an adornment to bulb culture, an asset to the trade, and an achievement of science.

In the hyacinths partial staminody, the partial metamorphosis of perianth leaves into stamens, has occurred. The three inner perianth leaves have become shorter and narrower. To the right and left the darker-colored tissue of the anthers is visible. At maturity this tissue bursts, and the yellow pollen appears. When microtome sections are made from these abnormally formed perianth leaves while they are young, one may clearly see the (sterile) pollen grains under the microscope.

The total number of mutations in hyacinths and tulips obtained after twenty-five years of X-ray treatment surpasses many times the number reported in publications.

Though even the smallest and least attractive deviations are important to science, only those varieties having a market value are raised on a large scale. Since 1940 a number of "X-ray tulips" without commercial value have been preserved by the author in a so-called Hortus Bulborum maintained for scientific research. Only one of the hyacinth mutations is still being propagated, since such culture is very expensive.

TEMPORARY MODIFICATIONS

In addition to permanent mutations, X-ray often results in modifications of a passing nature. During the first two years the X-rayed plants show, on the whole, a damaged aspect and an impaired growth (Pl. VI, Fig. 2). Though the amount of treatment has a definite influence on this phenomenon, such changes are bound to happen even with well-determined doses. The structure of the tulip bulb explains why plants appear to suffer most in the first two years after radiation. During the X-raying between July and November there exists in the bulb a large bud with a much smaller bud at its base. The first or final bud has progressed farthest and already contains the leaves and the flower for the next year.

[1] A yellow-white band above the blue spot at the base of the perianth leaves.

The smaller bud is a new starting point for the second year, with several small scales already split off. This bud also is reached by the X-rays and, when in the second year it grows into a plant, it still bears traces of the previous treatment. The buds which at the time of treatment had not developed show the mutated form, but develop more normally.

DIVISION HYPOTHESIS

The radiant day of April 24, 1937, will not easily be forgotten by the author. Dr. L. Randolph, of the Cornell University Agricultural Experimental Station, had asked permission to attend some experiments. In the laboratory he found a plant in full bloom; it was the tulip Prosperity, of which one half of the flower was dark red, whereas the other half was pure white.

To explain this result of X-ray treatment I proposed the division hypothesis (De Mol, 1942). The ordinary Prosperity has a pink flower. The process of division of the genes for pink had been hindered. When the mother nucleus divided into two daughter nuclei, these genes were kept in one daughter cell by the X-raying (duplication of genes) and consequently were not able to reach the other one (deletion). This daughter nucleus could not develop a pink anthocyanin; hence the white half of the flower. The first daughter nucleus, on the other hand, received all the genes for pink; hence the red half of the flower (cumulative effect). Many cases of bud mutation can be explained by duplication and deletion.

The origin of other modifications may be explained similarly. When the external conditions influence the cell in a uniform way, with the result that the division — albeit disturbed — proceeds equally, a modification lasting one or more years occurs. With an unequal influence and an inharmonious course of the division, cells with too many or too few chromosomes or chromosome fragments originate. This influence on the cytoplasm then causes the hereditary diversity.

SUGGESTIONS FOR X-RAY TECHNIQUE

The technique, experimentally developed since 1922, not only takes into account the doses, quality, and duration of the treatment, but also, and more especially, the phase and development rate of the bulb. This, of course, is closely related to the particular variety to be treated. During the first years tulips were exposed to various dosages of X-ray up to 1,000 roentgen units. After repeated experiments it was established that it was not wise to surpass 800 roentgen units.

The effects of the hardness of the rays showed plainest in Krelage's Triumph, in which the heaviest damage corresponded with the highest voltage. Full-grown large bulbs treated in August and September proved most satisfactory. They contain the greatest number of buds and young bulbs and, because of their dryness and hardness, are better protected against damage during transport and X-ray treatment.

If one wishes to bring out all the possible mutations and all the possible genetic changes in the bulbs that may result from one treatment, he should propagate them for at least four years.

SUMMARY

The history of mutation in tulips is reviewed, and the results of X-ray treatment since 1922 are summarized. Concurrent nonhereditary aberrations are discussed. A "division hypothesis" based on duplication and deletion is reviewed. Treatment in August and September with dosages not in excess of 800 roentgen units are most successful.

Amsterdam, The Netherlands

LITERATURE CITED

De Mol, Willem Eduard. 1929. Änderung der chromosomengarnitur durch Röntgenbestrahlung und Temperaturwirkungen (Retardation und Diversität). Zeitschrift für inductive Abstammungs- und Vererbungslehre, 54 : 363–367.

—— 1933. Die Entstehungsweise abnormaler Pollenkörner bei Hyazinthen, Tulpen und Narzissen. Cytologia, 5 : 31–65.

—— 1937. L'Effet de l'irradiation des oignons a fleur par les rayons-X (de jacinthes et de tulipes). Revue Technique Philips, (11)2 : 321–328.

—— 1942. Die früher blühenden somatischen Tulpenmutanten (Untersuchungen, 1940–1941) und die Teilungshypothese. Die Gartenbauwissenschaft, 17 : 106–132.

Red Darwin Tulip William Pitt (right) and its X-ray mutation, Sparkling Pitt
(Photograph by J. Agterberg)

X-ray mutations in petals. Upper: Red, white and yellow sectors in a petal of Darwin Tulip Alland Pierson. Middle: Lighter sector in Darwin Tulip Bartigon. Lower: Small red sector in Mon Tresor. (From water colors by E. Loudon. Photograph by George Schneider)

FIG. 1

FIG. 2

White X-ray mutations in half of Parrot Tulip Red Champion (Fig. 1) and in half of Early Tulip Peach Blossom (Fig. 2). From water colors by E. Loudon. (Photographs by George Schneider)

Variegated leaf of Parrot Tulip Gemma
(Photograph by C. A. van der Gen)

FIG. 1

FIG. 2

FIG. 1. Single Early Tulip General De Wet. Drawing E. Loudon
FIG. 2. X-ray mutation form General De Wet to Parrot form

FIG. 1. Giant X-ray Tulip Red Champion
Photograph by J. Agterberg

FIG. 2. Aspect of damaged tulips the first year after X-ray treatment. (Photograph by Agterberg)

THE EFFECT OF SIX VITAMINS UPON THE GROWTH OF *FUSARIUM SOLANI* IN A SYNTHETIC MEDIUM

SISTER MARY VIRGIL GHERING

THE culture of *Fusarium solani* used in this investigation was identified by Professor William C. Snyder,[1] of the University of California. It was carried on a potato-dextrose agar. Spore suspensions were made with a 5 per cent solution of peptone in normal saline, and were stored in a refrigerator. It had been reported to the author that such suspensions could be kept indefinitely under these conditions, but germination was observed after a few weeks, when spore counts were made with a hemocytometer.

Every component of the nutrient medium employed was chemically defined. A mixture of amino acids replaced the "vitamin-free" casein hydrolysate, which is used in many of the microbiological procedures for the assay of various vitamins. Hydrolysis of casein is usually effected by heating with strong acids, but it is doubtful whether the reaction is ever complete. The products of hydrolysis include, besides the alpha-amino acids, ammonia and humin. Tryptophane is lost in the insoluble humin fraction, while tyrosine is involved in the soluble brown humin. If hydrochloric acid is used, some cystine is destroyed in the subsequent removal of chloride ions by means of silver salts. Ordinary technical casein contains a considerable amount of vitamins. To remove completely traces of niacin and other vitamins, the casein is subjected to treatment with special activated carbons. The adsorption of amino acids and peptides by activated charcoal has been reported. It is probable, therefore, that casein hydrolysate varies from lot to lot according to the content of the original casein, the extent of hydrolysis, and the amount of treatment by norite for the removal of vitamins.

[1] Snyder and Hansen (1945) have proposed a revision of the taxonomy and nomenclature of the Fusaria. Their system is based upon evidence of variability, occurring in the progeny of cultures isolated from single spores of many so-called species in the genus.

Some microorganisms are able to synthesize all the amino acids they require from a single one or from asparagine. They display better growth, however, when a mixture of amino acids is furnished in equivalent amount in the culture medium. Robbins and Ma (1945) suggest that substances which are supplied in the medium may be incorporated into fungal proteins more rapidly than those which must be synthesized by interconversions in living tissue. Many fungi require a complex nitrogen source. The nitrogen requirements of these organisms are determined by omitting one component at a time from a mixture of amino acids which supports growth. When all the nonessential components are omitted at the same time, however, growth seldom results. Under these conditions additional amino acids are indispensable. Snell (1946) recommends the use of a complete assortment of amino acids for both vitamin and amino-acid assay.

The optimal combination of amino acids is not known. Since casein is the protein having most satisfactory nutritive properties, a synthetic casein hydrolysate has been widely used. However, it is not certain that casein supplies all the amino acids in ideal proportions. It is considered likely that the minimum protein requirement, as determined by the optimal combination of amino acids, may prove to be much lower than that secured with casein.

The amino-acid requirements of *Lactobacillus arabinosus* and *L. casei* were investigated by Hyma (1946). He used Snell and Wright's medium (1941) for the assay of niacin and Gladstone's medium (1937) as points of departure. Hyma tried more than five hundred combinations of amino acids, and as a result of his work formulated the amount of each one which gives maximum growth of the test organisms. This was the mixture used in the present investigation. It included the twenty commonly occurring amino acids plus the purine bases, adenine, guanine, and xanthine; the pyrimidine base, uracil; and asparagine. Asparagine has been reported to accelerate growth, especially in the earlier stages. The other substances have been shown to be accessory growth factors for the lactic-acid bacteria. Because of the fundamental unity which underlies all biochemistry, it is very likely that they act as growth-promoting substances for other organisms as well.

The stock mixture of amino acids was kept in a tightly stoppered brown bottle. As compared with casein hydrolysate, it is a very convenient nitrogen source. Casein hydrolysate contains no preservative; therefore the solution remaining in the bottle must be sterilized each time the stopper is removed. We did find it possible, however, to withdraw the

solution with a sterile hypodermic needle and thus eliminate the need for repeated sterilization.

A comparison of the relative values of asparagine, casein hydrolysate, peptone, and the amino-acid mixture is indicated in the tubes of Figure 1.

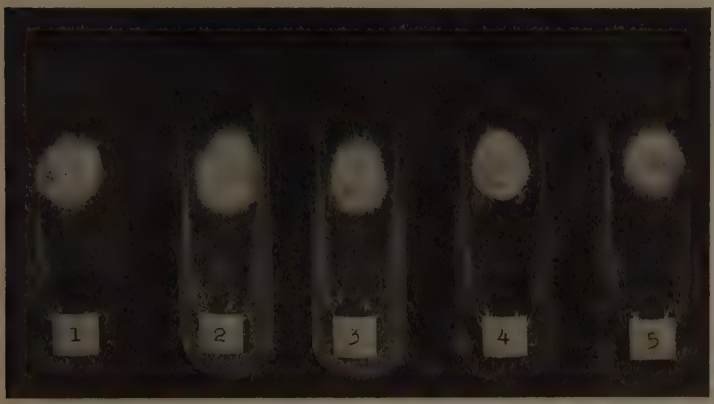

FIG. 1. A comparison of the effects of nitrogen from various sources upon the growth of *Fusarium solani* after six days on an agar medium containing sugar, salts, and asparagine. Tube 1 contained 8 mg. neopeptone; tube 2, 60 mg. casein hydrolysate; tube 3, 360 mg. casein hydrolysate; tube 4, 62 mg. amino-acid mixture. No addition was made to tube 5

Each tube contained eight milliliters of an agar medium made up of sugar, salts, and asparagine. To tube 1 was added peptone; to tube 2, casein hydrolysate in lesser concentration; to tube 3, casein hydrolysate in greater concentration; to tube 4, the amino-acid mixture. No addition was made to tube 5. The asparagine medium yielded a thin, spreading growth on the surface of the agar. The amino-acid mixture was not greatly inferior to the natural products when either extent or character of growth was considered. The edges of the colony, however, were more sharply defined. Casein hydrolysate in greater concentration gave less good growth than casein in lesser concentration. The latter produced the best growth if judged on the basis of diameter of colony, but peptone excelled in character of growth.

In this experiment it was observed that a deep-red pigment suffused throughout the peptone medium; but in the medium containing the amino-acid mixture not the slightest trace of pigment was produced. The asparagine medium acquired a pinkish-orange cast, and the casein-hy-

drolysate medium became pink. A deeper shade of color developed in the medium which contained the lesser concentration.

Subsequently the fungus was cultured on an agar medium with the amino-acid mixture as the nitrogen source and in the presence of single vitamins. Little difference was observed so far as colony diameters were concerned; but the presence of the vitamins, except p-aminobenzoic acid, seemed to encourage the spread of hyphae in the medium. No pigment was observed in any of the tubes except those which contained biotin and i-inositol. In the presence of these substances the medium became a deep-rosy red. It does not seem rash to suggest that the production of pigment in the media containing peptone and casein hydrolysate was due to the presence of vitamins in them. Many workers have reported the occurrence of various vitamins in peptone. After several weeks a faint-pink color was observed in all the tubes, except that containing p-aminobenzoic acid. If the production of pigment is evidence of the presence of biotin or i-inositol in the medium, their occurrence may be explained by the ability of the fungus to synthesize them at a very slow rate.

The initial pH of the medium seemed to have little effect upon the growth of the fungus. According to Wollenweber and Reinking (1935), this phenomenon is not unknown among the Fusaria. A potassium-citrate buffer was employed. The presence of the buffer in the medium resulted in a marked increase in growth, which seemed to be out of proportion to its effect upon the pH of the medium. It has been reported that beta-alanine is active only in the presence of biotin, thiamin, asparagine, and glutamic acid. Organic acids, however, and citric acid in particular, can replace the asparagine and glutamic acid. Citric acid reduces the optimal concentration of beta-alanine to a tenth of the usual amount.

The determination of the effect of a vitamin upon the growth of the fungus was practically the same procedure that is employed in microbiological assays of vitamins. A basal medium was prepared containing all the vitamins except the one under test. The sugar, salts, and amino acids were dissolved in distilled water over a steam bath. Appropriate amounts of the trace salts, buffer, and vitamin solutions were added, and the volume was brought to one liter. The solution was divided into ten portions, and the vitamin under test added in amounts calculated to give the desired concentrations. Biotin and folic acid were added after sterilization. The stock solutions of these vitamins were made up with sterile distilled water, and were stored in a refrigerator.

The volume of nutrient solution employed for culturing the test organism was 25 milliliters in 125-milliliter Erlenmeyer flasks. A standard

loop was used for inoculation. One loopful of the spore suspension was the standard inoculum for both the liquid cultures and the agar slants. The cultures were agitated after inoculation, but were incubated without agitation for six days at 27° C. Growth was determined by autoclaving the cultures, filtering, and drying the mycelial mats to constant weight at 100° C. The filter papers had previously been numbered, folded, and dried on watch glasses to constant weight. They were stored in a desiccator.

No vitamin proved to be essential for growth of *Fusarium solani*. Biotin, folic acid, and calcium pantothenate, however, stimulated growth. When the average dry weight of mycelium in milligrams was plotted against the concentration of these vitamins in gamma per milliliter, S-curves were obtained. The stimulatory effect of these vitamins may be due to the inability of the fungus to synthesize them as rapidly as they can be used if furnished from the outset in the culture medium.

It is not surprising that biotin should exert a beneficial effect upon growth. Barnett and Lilly (1947) secured some evidence that the ratio of the weight of biotin in the medium to the weight of mycelium at the time of perithecial formation must be at least one part to twenty million for perithecial formation to occur. Folic acid may be synthesized by microorganisms in the digestive tract. Persons getting sulfa-drug treatment occasionally develop a white blood cell deficiency. The same type of blood disorder appears in persons who have been deprived of folic acid. Elvehjem (1943) suggests that the sulfa drugs may destroy the microorganisms in the intestinal tract which ordinarily manufacture folic acid. In view of the almost universal occurrence of pantothenic acid in tissue extracts, the slightly beneficial effect of calcium pantothenate is not difficult to understand.

These experiments indicate that pyridoxine and riboflavin are without effect upon the growth of *Fusarium solani*. Pyridoxine was discovered in studies of animal nutrition, and with the exception of a few species of Ceratostomella, fungi do not require it. Hawker (1944) stated that riboflavin, niacin, and the fat-soluble vitamins had not so far been reported essential for the growth of any fungus, although some species were known to synthesize one or more of them. It may be that they play a rôle in the biosynthetic processes of fungi, but that all fungi are able to synthesize them.

Niacin seemed to exert an injurious effect upon growth, which was greater in the absence of niacin than under any other condition of the experiment. This might be due, however, to changes in the composition

of the medium during heat sterilization. Knight (1945) affirms that heating asparagine and glutamic acid together in the presence of manganese and air yields nicotinamide, which was identified by isolation and characterization. Since these substances were present in the medium, it may be that the harmful effect of niacin was due to its presence in excess.

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LITERATURE

Barnett, H. L., and Lilly, V. G. 1947. The Effects of Biotin upon the Formation and Development of Perithecia, Asci, and Ascospores by *Sordaria fimicola* Ces. and de Not. Am. Journ. Bot., 34 : 196–204.

Elvehjem, C. A. 1943. Vitamins. Science, 97: Supplement No. 2518 (12).

Fildes, P., and Richardson, G. M. 1937. The Nutrition of *Staphylococcus aureus*. Sulphur Requirements. Biochem. Journ., 18 : 301.

Gladstone, G. P. 1937. The Nutrition of *Staphylococcus aureus*. Nitrogen Requirements. Brit. Journ. Exper. Pathol., 18 : 322–328.

Hawker, L. E. 1944. Fungi and Vitamins. Sci. Journ. Roy. Coll. Sci., 14 : 70.

Hyma, A. M. 1946. A Synthetic Medium for Microbiological Assay of Riboflavin, Pantothenic Acid, Biotin, Nicotinic Acid, Pyridoxine and Folic Acid. A Ph.D. thesis on file at Michigan State College.

Knight, B. C. J. G. 1945. Growth Factors in Microbiology. Vitamins and Hormones, 3 : 185, 228.

McIlwain, H. 1939. Pantothenic Acid and the Growth of *Streptococcus haemolyticus*. Biochem. Journ., 20 : 330–333.

Robbins, W. J., and Ma, R. 1945. Growth Factors for *Trichophyton mentagrophytes*. Am. Journ. Bot., 32 : 522.

Schmidt, C. L. A. 1945. Chemistry of Amino Acids and Proteins. Second Edition, pp. 124–129, 993, 1047. Charles C. Thomas, Publisher. Springfield, Illinois.

Schopfer, W. H. 1943. Plants and Vitamins, p. 132. Chronica Botanica Company. Waltham, Massachusetts.

Snell, E. E. 1946. Microbiological Methods in Amino Acid Analysis. Annals of the New York Academy of Sciences, 47 : 163–165.

—— and Wright, L. D. 1941. A Microbiological Method for the Determination of Nicotinic Acid. Journ. Biol. Chem., 139 : 675–680.

Snyder, W. C., and Hansen, H. N. 1945. The Species Concept in *Fusarium* with Reference to Discolor and Other Sections. Am. Journ. Bot., 32 : 657–666.

Williams, R. J., Lyman, C. M., Goodyear, G. H., Trusdail, J. H., Holiday, D. 1933. "Pantothenic Acid," a Growth Determinant of Universal Biological Occurrence. Journ. Am. Chem. Soc., 55 : 2927.

Wollenweber, H. W., and Reinking, O. A. 1935. Die Fusarien, p. 274. Paul Parey. Berlin.

SOME NUTRITIONAL ASPECTS OF THE PISTILLATE CONDITION IN RAGWEEDS*

KENNETH LESTER JONES

THE giant ragweed, *Ambrosia trifida* L., is monoecious with pistillate flowers in the leaf axils and staminate flowers in conspicuous racemes. I have not seen deviations from this pattern in nature.

In 1945 I collected in one locality along the Huron River near Ann Arbor, Michigan, seed infested with larvae. The following spring sixty-four plants were grown from them, eight of which bore some pistils in the racemes. The leaves were misshapen and blotched with yellow areas.

I was then carrying on photoperiod experiments and found, as had Mann (1942), that plants of *A. trifida* given six hours of light a day produce pistillate structures in place of staminate. The seed that occasionally form from such abnormally developed pistils are about one third the usual size. It happened that the first that I succeeded in germinating grew into dwarfed plants with misshapen leaves (Pl. I, Fig. 1) and pistillate racemes.

These observations suggested that sex reversal may be brought about by a deficiency of food in the cotyledons. A simple experiment was therefore run to test the effect of the removal of cotyledons from normal seedlings. Carefully selected seed were sown on June 11, 1947, and the plants were grown in the greenhouse under normal light conditions of the summer season. The results are given in Table I.

The occurrence of a few aberrant plants in the control was hardly expected, since I had encountered none either in nature or under ordinary conditions of cultivation. No significant increase in sex reversal was brought about by the removal of cotyledons or plumule. (Plate I, Figure 2, shows some of the altered racemes.) I had previously reported (1936) that in Form 2 of *Ambrosia elatior* excision of the plumules increased the pistillate plants by 6.4 per cent.

In the meantime additional information has been obtained on the

* Paper from the Botanical Gardens and the Department of Botany, University of Michigan, No. 894.

offspring from seed of sexually reversed plants from the photoperiod work. Such minute seed do not, as first supposed, ordinarily form individuals with pistillate racemes. To date (April, 1949), 230 plants have been grown and only 7 have borne racemes with pistillate flowers. Dwarfing is likewise not the rule, since only 6 plants showed this condition, 3 being pistillate.

Pruning and defoliating of plants in which floral primordia have formed appreciably increases the percentage of sex reversals (Table I).

TABLE I

THE FORMATION OF RACEMES WITH PISTILLATE HEADS IN AMBROSIA TRIFIDA

Material	No. of plants	With racemes	Racemes pistillate	Percentage pistillate
Control	100	96	1	1
Cotyledons removed	100	91	3	3
Plumule and cotyledon removed	102	85	2	2
Pruned	50	46	6	12
Defoliated	109	48	14	12
Defoliated, sucrose	71	24	5	7

The pruned plants were cut back to bear only one leafy branch. The plants that were defoliated were grown as individuals with two equal, opposite branches; one branch was left intact, and the other was completely defoliated when floral primordia appeared. As Table I indicates, over 50 per cent of the defoliated branches failed to produce racemes. The foliated branch always gave rise to staminate racemes.

A second set of defoliated plants was sprayed with a 10 per cent aqueous solution of sucrose in which "carbowax" was used as a spreader and sulphanilamide added to keep down the growth of fungi. The percentage of reversals to the pistillate condition was slightly less than that of the corresponding set not receiving sucrose, but there was a considerable increase in racemeless individuals.

Tampering with the nutrition of Ambrosia plants in the early vegetative stage seldom leads to a sex reversal. This is borne out by the present work and by previous reports of negative results when mineral nutrients were added to the soil (Jones, 1936) and when plants from ten to twenty days old were exposed to short days (Jones, 1947).

Once floral primordia are initiated, several external agents of unlike

nature bring about an alteration from staminate to pistillate structures. Short-day treatment is particularly effective, but defoliation and the application of chemicals to the stem apex may give results. I have had some positive results by using indole acetic, phenylacetic, and α estradiol propionate (Pl. II, Figs. 1–2). The change from the pistillate condition of the lateral flowers to the staminate is rare, but two instances were noted (Jones, 1947).

The fact that defoliation alone is sufficient to produce sex reversal is a vexation to the experimenter studying the effects of photoperiodism. If he takes a two-branched foliated plant and gives one branch short-day exposures, the treated branch will usually form some pistils in its raceme, whereas the branch receiving the normal photoperiod of late summer will be staminate. If the untreated branch is defoliated, it will very likely become pistillate, but it is impossible to ascertain whether this reversal in a given specimen is the result of defoliation or of a transfer of some stimulus from the short-day branch.

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LITERATURE CITED

Jones, K. L. 1936. Studies on Ambrosia. II: Effect of Certain Environmental Factors on Floral Development of *Ambrosia elatior*. Bot. Gaz., 98 : 296–306.

—— 1947. Studies on Ambrosia. IV. Effects of Short Photoperiod and Temperature on Sex Expression. Am. Journ. Bot., 34 : 371–377.

Mann, L. K. 1942. Effects of Photoperiod on Sex Expression in *Ambrosia trifida*. Bot. Gaz., 103 : 780–787.

PLATES I–II

FIG. 1. Malformed leaves of *A. trifida* pistillate plants. The parent plants underwent sex reversal from short-day treatments

FIG. 2. Pistillate racemes of *A. trifida* from plants from which the cotyledons had been removed

FIG. 1. Branch of *A. elatior* bearing staminate racemes (control)

FIG. 2. Branch of *A. elatior* on the same plant and opposite to the one shown in Figure 1. This cluster of four racemes, three of which show some pistillate structures, developed from a stem apex to which α estradiol propionate had been applied

SOME ECOLOGICAL ASPECTS OF THE ACRASIALES IN AND NEAR MADISON, WISCONSIN

EUGENE D. KITZKE

INTRODUCTION

IN THE Myxomycetes there is a relatively small group known as Acrasiales. They are strikingly different from true Myxomycetes in that the individual myxamoebae retain their plasma membranes, keep their individuality, and form a pseudoplasmodial stage. The plasmodial stage of the true Myxomycete differs, of course, in that it has no individual cells and is a naked mass of multinucleated cytoplasm.

Since the group as now known is a small one, a concise study of its distribution in a limited area can be made in a summer's work. Sixty soil samples from various localities within a twenty-five mile radius of Madison were tested. In addition, an attempt was made to find significant correlations between certain measurable factors of the soil and the presence of the organisms. The soil factors of pH, the percentage of organic matter, and the colloid percentage were determined for each sample. The plant associations of each soil source were also considered as variable factors.

MATERIALS AND METHODS

Of sixty soil samples thirty-six yielded members of the Acrasiales. From these, four species were identified. Two belonged to the genus *Dictyostelium* and two to *Polysphondylium*.

Representative field samples of grassland, marshland, sand dunes, shrub areas, and humus from various stands of hardwood forests were collected in sterile 60 c.c. bottles. They were taken to the laboratory, where they were processed almost immediately by various soil-analysis procedures (to be described later).

METHOD OF ISOLATION

The soil sample was poured out on a fresh paper towel, and particles or crumbs of it selected at random were placed on petri dishes of soil

agar (Singh, 1946). Ten plates were prepared from each sample in order to permit a calculation of the distribution frequency percentage for the organisms. Incubation was carried out at room temperature (summer temperatures ranging from 22° to 26° Centigrade) for four days, after which the plates were examined for fruiting structures of the fungus.

Plates containing the fruiting structures of the Acrasiales were set aside an extra day for further development to permit heavier growth. The next day the remaining plates, hitherto blank, were reëxamined to determine whether any slower-growing forms had appeared, a thing that rarely happens. Plates bearing the desired organisms were then transferred by inoculation of terminal sorus spores to petri plates of mannite agar (*Difco Manual*, 1937) already supporting a streak growth of *Escherichia coli*.

The bacterial population of the streak afforded abundant food for the Acrasiales, and they attained luxuriant growth in this mixed culture. Often the transfer from soil agar carried along with it contaminants, the growth of which was eliminated by a second transfer. The organism, when finally isolated into a "pure-mixed"[1] culture (Raper, 1937), was transferred to a mannite agar slant for stock culturing and identification. Since the Acrasiales require bacteria for food, they must be kept in such a culture.

Because soil is a rich source of a great variety of fungi, the medium originally used to isolate the Acrasiales had to be one that could limit the number of contaminants. Experience with mannite agar has shown that heavy growth of unwanted forms of fungi occur on the plates and may obscure the more delicate Acrasiales. This could be obviated if precautions were taken to incubate the plates at 15° to 16° Centigrade for a two-week period; but this technique was impracticable in the present study because of summer temperatures and lack of incubator space.

The use of soil agar as the initial isolating medium greatly expedited matters, for Acrasiales, if present, appeared after four days' incubation at room temperature. The low nutrient value in this medium prevented the normal overgrowth that occurred in the comparatively rich mannite agar. The soil-extract agar was prepared after Hesseltine (paper in preparation). Its composition was as follows:

[1] The term "pure-mixed" as applied to a culture refers to the growth of some suitable bacterium and a member of the Acrasiales, each in pure culture, in the same dish or tube.

Agar agar	15 gm.
K_2HPO_4	0.5 gm.
Glucose	1 gm.
Soil extract[2] ...	100 c.c.
Tap water	900 c.c.

The original purpose for which this medium was designed was to isolate and culture mucors without getting a heavy overgrowth of contaminants or of the mucors themselves. Hesseltine noticed that it also lent itself rather consistently to supporting the growth of members of the Acrasiales. Thus it was adopted by the writer.

TECHNIQUE OF SOIL ANALYSIS

The soil samples were processed immediately after collection. The pH was determined by means of a Beckman pH meter. The approximate percentages of organic matter in them were ascertained by the use of the ash technique of Schollenberger (1931). When the margin of error due to carbonates and other compounds that may have been oxidized has been taken into consideration, the values obtained may be considered approximations.

The colloidal percentage was determined for each sample by the use of the Cenco-Wilde[3] test, known as the hydrometer field test.

ORGANISMS ISOLATED

Four species belonging to two genera were isolated. Two, *Dictyostelium mucoroides* and *Polysphondylium violaceum,* occurred very commonly. In a small number of plates *Polysphondylium pallidum* appeared, a not uncommon species, according to Raper (1940), and *Dictyostelium minutum,* which is perhaps rare, since it has been reported infrequently in the literature.

Polysphondylium violaceum

P. violaceum has been described in an earlier paper by Kitzke (1950).

Dictyostelium mucoroides Brefeld

The measurements of the spores of *D. mucoroides* Brefeld, 3–5 × 5–8 μ, are somewhat larger than those given by Olive (1902); he does

[2] The soil extract was prepared by heating one kilogram of soil with one liter of tap water in an Arnold sterilizer for one hour, after which it was filtered two or more times until clear and then autoclaved for one hour on two successive days.

[3] Cenco-Wilde Soil Colloid Tester No. 28250.

state, however, that the range is great. The diameters of the sori range from 100 to 600 μ, which agrees with Olive's measurements. The heights of the sorocarp differ greatly, ranging in many from 5 mm. in some to well over 1 cm.

Dictyostelium minutum Raper

The spores of *D. minutum* measured 3–3.5 × 5–7 μ. An extensive study of their sizes revealed none outside this range, which is identical with that given by Raper (1941). The heights of the sorocarp ranged from 350 to 750 μ. The sori exceeded very little the largest diameter in the range (75 to 150 μ) listed by Raper. In spite of the ample food supply and the frequent transfers the growth of the organism was consistently small, which indicates that the species is different from any described, except *minutum*.

Polysphondylium pallidum

P. pallidum, described by Raper (1940) as of common occurrence, was found only infrequently in our samples. The measurements of the isolate, which correspond closely with those listed by Olive, are as follows: spores, 3.5–4 × 7–8 μ; terminal sori, 50–90 μ in diameter; lateral sori, 30–40 μ in diameter; sorocarp, 300–400 μ tall.

CORRELATIONS

The sites of collection, all within a twenty-five-mile radius of Madison, are listed in Table I.

TABLE I

SOURCES OF THE SOIL SAMPLES

Site	*Location*
Grady tract	University Arboretum
Stewart's woods	Dane County
Bascom woods	University campus
Wollein's woods	Jefferson county
Willow drive	University campus
Grasslands	Forest Products Laboratory Road
Pine barrens	Arena
Prairie	Lake Mills

The pH values of the soils tested were within a narrow range and afforded little variation to enable me to detect growth patterns. When,

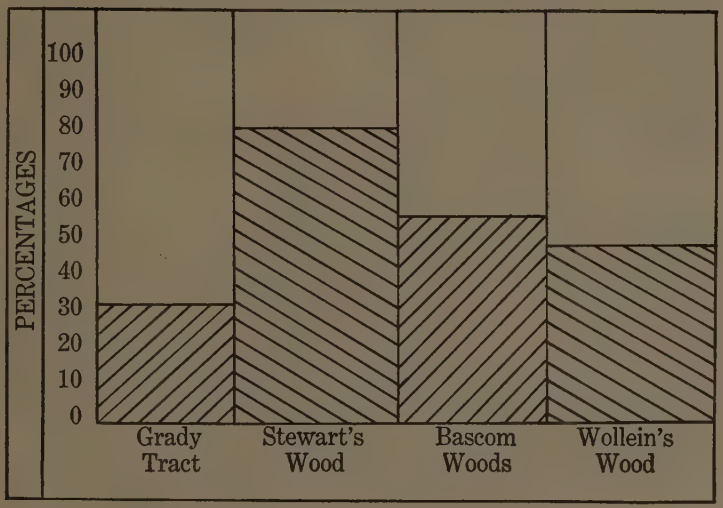

FIG. 1. Frequency percentage of Acrasiales in four quadrats

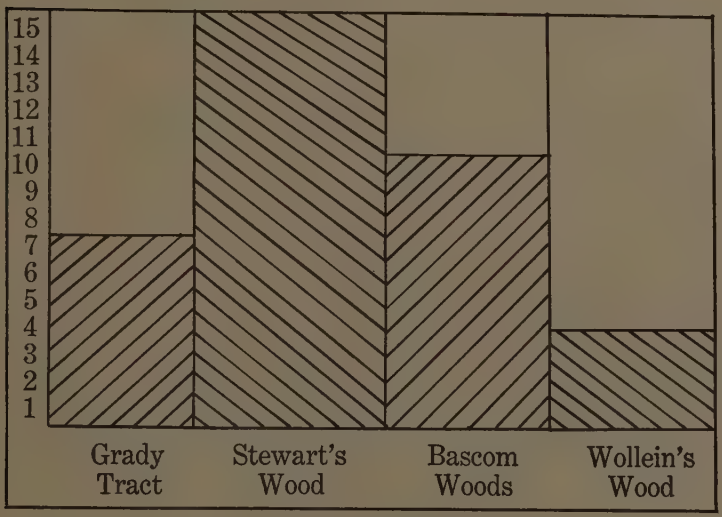

FIG. 2. Number of shrub species

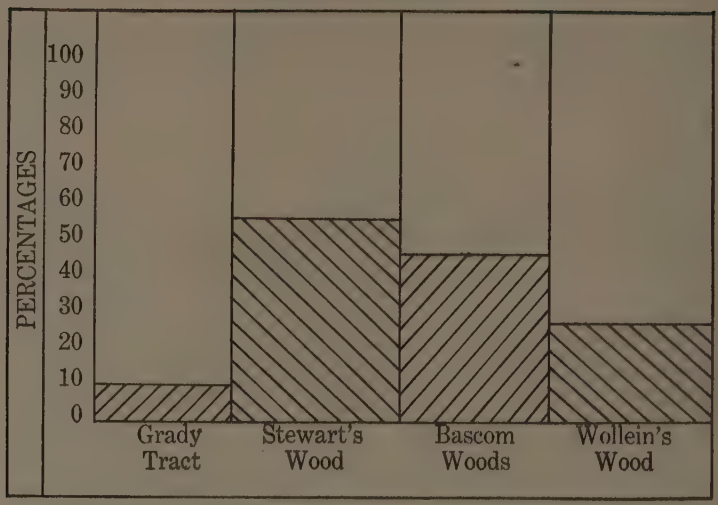

FIG. 3. Frequency percentages of herb species in four oak quadrats

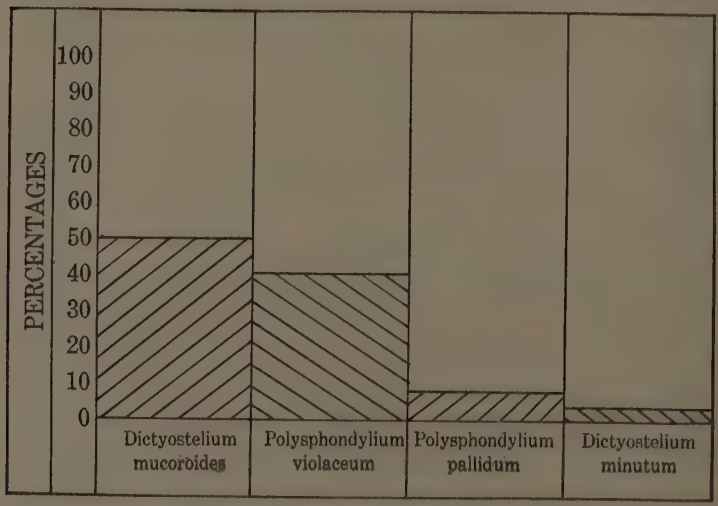

FIG. 4. Relative percentages of Acrasiales isolated

however, a large number of pH values below 6 were found, which, according to Raper (1939), is definitely not optimum, there was less growth of Acrasiales.

Soil colloid percentages and minimum organic percentages do not seem to have any obvious correlation with the presence of the organisms. In general, however, with the increase in the percentage of organic matter there is an increase in the frequency percentage of Acrasiales.

Figure 1 shows the frequency percentages of Acrasiales in the four quadrats which were studied thoroughly from an ecological viewpoint. Stewart's woods has the highest frequency percentage of Acrasiales and also the heaviest occurrence of herbs and shrubs. In fact, the graphs showing the data for the herb and shrub species (Figs. 2–3) closely resemble those for the occurrence of Acrasiales in these quadrats. This may be due to the greater number of bacteria decomposing the herb and shrub litter, which accumulates more rapidly in Stewart's woods than in Bascom woods, the Grady tract, or Wollein's woods. Thus an increase in the number of bacteria provides a greater food supply for the Acrasiales, and hence enables them to attain more uniform distribution.

Of the sixty samples taken and tested 60 per cent contained members of the Acrasiales. Only 40 per cent showed a complete absence of them or failure of the organisms to develop from them when inoculated with soil. The percentages of the different species found in the successful plates are given in Figure 4.

The total number of isolations was 157, composed of the following species: *Dictyostelium mucoroides*, 76; *Polysphondylium violaceum*, 60; *P. pallidum*, 12; and *D. minutum*, 9. These figures seem to be indicative of their relative distribution in the soils tested and confirm in general the reports of other workers (Raper, 1940; Olive, 1902).

SUMMARY

Members of the Acrasiales were isolated from soil samples taken at random within a twenty-five-mile radius of Madison, Wisconsin. The experiments show that soil agar provides an excellent isolating medium and that mannite agar used in connection with "pure-mixed" cultures is a good medium for stock culturing. The Acrasiales occurred in 60 per cent of the sixty soil samples tested.

No correlations for colloidal percentages were noted. With respect to the percentage of organic matter of the soil samples there was found to

be an increase of Acrasiales, with an increase of organic material in the soil.

The factor of pH does not seem to affect the frequency of occurrence of Acrasiales, mainly because of great variability in soil types. There seems to be a lower frequency percentage of Acrasiales, however, in plates inoculated from soils near pH 5 and 8.

Four species of Acrasiales were isolated representing two genera. Three species, *D. mucoroides, P. violaceum,* and *P. pallidum,* were reported common by other workers. One, *D. minutum,* first isolated by Raper (1941), appears to be relatively rare. *P. pallidum* also occurs only infrequently in this study.

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LITERATURE CITED

Difco Manual. 1937. Difco Products Co., Detroit, Mich.

Hesseltine, Clifford W. A Revision of the Mucorales Based Especially upon a Study of the Representatives of This Order in Wisconsin. (In preparation.)

Kitzke, Eugene, D. 1950. Two Members of the Acrasieae Isolated in Milwaukee County, Wisconsin. Pap. Mich. Acad. Sci., Arts, and Letters, 34 (1948): 13–18.

Olive, E. W. 1902. Monograph of the Acrasieae. Proc. Bost. Soc. Nat. Hist., 30: 451–513.

Raper, Kenneth B. 1937. Growth and Development of *Dictyostelium discoideum* with Different Bacterial Associates. Journ. Agric. Res., 55: 289–316.

—— 1939. Influence of Culture Conditions upon the Growth and Development of *Dictyostelium discoideum. Ibid.,* 58: 157–198.

—— 1940. Communal Nature of the Fruiting Process in the Acrasieae. Am. Journ. Bot., 27: 436–448.

—— 1941. *Dictyostelium minutum,* a New Species of Slime Mold from Decaying Forest Leaves. Mycologia, 33: 633–649.

Schollenberger, C. J. 1931. Determination of Soil Organic Matter. Soil Sci., 31: 481–486.

Singh, B. N. 1946. Soil Acrasieae and Their Bacterial Food Supply. Nature, 157 (3979): 133–134.

THE EFFECT OF HORMONE ON BARK RENEWAL IN *HEVEA BRASILIENSIS* *

THOMAS J. MUZIK

BARK renewal of *Hevea brasiliensis* Muell. Arg., the Pará rubber tree, after tapping has been the subject of considerable investigation. It has been reported that various substances increase the rate of renewal. Beeley and Baptist (2) found that palm oil improved it from 25 to 50 per cent over a three-month period, although, strangely enough, Baptist (1) obtained no increase one year after applying indolyl-acetic acid, and he ascribed this lack of effect to the weak concentrations used, 1 : 1,000, 1 : 5,000, and 1 : 10,000 in lanolin. Other workers have reported that a number of substances are more or less effective, namely, mineral oil (2), coal tar (9), cow dung and clay mixture (1), tar (10), a solution of MgCl and NaCl in water, and distilled water alone (6). Sharp (8) noted that healing of large stumps was promoted by treatment with cow dung and Hessian cloth, whereas linseed oil and zinc oxide had no effect, and an asphalt-kerosene mixture retarded healing. Gandrup (4) claimed that tar had no effect on bark renewal.

Since many of these substances probably contain hormones and since the results reported above conflict, the author undertook the experiments here described.

MATERIALS AND METHODS

These experiments were carried out on the Firestone Plantations in Liberia, West Africa, from December, 1945, to March, 1946, the "dry season" in that part of the tropics. The clone Avros 152 was chosen because the bark is reasonably free from knots or ridges, and natural renewal is relatively slow. Only the lower half of each panel was treated; the upper half served as the control (Pl. I). Thus each tree was used for

* Papers from the Department of Botany, University of Michigan, No. 895.

This work was done while the author was a member of the Research Department of the Firestone Plantations Company, Harbel, Liberia. It is published with the permission of the Company.

both treatment and control, and the relative growth rates of treated and untreated zones of the same panel could be measured. This method eliminated variation due to different growth rates of trees, a factor which may well have influenced the results reported above. As La Rue (7) has pointed out, the rate of renewal is affected greatly by many environmental conditions, such as soil and the tapping system, as well as hereditary factors.

Two hormones were tested, both in lanolin, indole-3-acetic acid in concentrations of 1 : 1,000, 1 : 5,000, and 1 : 10,000, and indole-butyric acid in concentrations of 1 : 1,000, 1 : 5,000, and 1 : 10,000. The effect of pure lanolin was also tested. Five trees were treated with each concentration of hormone daily, immediately after tapping. The hormones were applied in the same manner as panel fungicides, that is, with a small brush and directly on the bark tapped on the same day. Bark thickness was measured at monthly intervals, by means of a standard bark gauge. An average of three measurements was taken at each reading, although the variation in thickness was considerably less in this clone than that reported by Haigh (5) for certain seedling trees.

These trees were planted as seedling stumps in 1928 and grafted in 1933, when four to five years old. They were first tapped in 1939 on a half-spiral system, then placed on a full spiral from July, 1942, to March, 1943, and returned to the half spiral in March, 1943. The panel was about twenty inches from the graft union, and the average girth at sixty-five inches above the union was 61.7 cm. The tapping cut was at an angle of 30° from left to right and was made from the upper side down toward the cup (Pl. I). An especially good worker was assigned to these trees to insure even tapping. The bark was removed to a depth of one millimeter from the cambium. The trees were tapped on alternate days, and one fifteenth of an inch[1] of bark was excised at each operation. Frequent inspections of the trees were made.

EXPERIMENTS WITH INDOLE–3–ACETIC ACID

Indole–3–acetic acid was used in the first treatments. It was applied daily in concentrations of 1 : 1,000, 1 : 5,000, and 1 : 10,000 in lanolin. Bark thickness was measured at monthly intervals for three months after treatment. The initial thickness was one millimeter. The depth of renewed bark after treatment is shown in Table I.

[1] Note the two systems of measuring; the depth of tapping is invariably measured in the metric system and the amount of bark removed, in the English system.

TABLE I

THE EFFECT OF INDOLE-3-ACETIC ACID ON BARK RENEWAL

In all these experiments the lower half of the panel was treated and the upper half served as a control.

Tree	Bark thickness in millimeters		
	First month	Second month	Third month
LINE A. Concentration 1 : 1,000			
1 Treated	7.2	7.8	7.8
Control	6.7	6.8	7.0
2 Treated	8.3	9.2	9.3
Control	6.2	6.3	7.2
3 Treated	8.3	9.6	11.0
Control	5.7	6.9	8.5
4 Treated	8.7	9.9	10.5
Control	6.5	7.0	7.8
5 Wind-damaged	..	..	..
Average:			
Treated	8.13	9.13	9.65
Control	6.28	6.75	7.63
Difference	1.85	2.38	2.02
Increase	29.5%	35.3%	26.5%
LINE B. Concentration 1 : 5,000			
1 Treated	7.5	9.5	9.6
Control	7.7	7.8	8.0
2 Treated	8.1	9.1	9.2
Control	4.8	6.0	6.4
3 Treated	9.5	10.8	10.8
Control	6.1	8.5	8.7
4 Treated	7.3	7.4	9.0
Control	5.5	5.9	6.2
5 Treated	6.1	7.1	7.3
Control	6.1	6.5	7.2
Average:			
Treated	7.70	8.78	9.18
Control	6.04	6.94	7.30
Difference	1.66	1.84	1.88
Increase	27.5%	26.5%	25.8%
LINE C. Concentration 1 : 10,000			
1 Treated	7.2	10.0	10.3
Control	6.0	7.7	8.0
2 Treated	6.9	7.0	7.5
Control	5.1	6.1	6.3
3 Treated	7.7	9.0	9.2
Control	6.7	7.1	7.5
4 Treated	8.0	8.1	8.6
Control	5.7	6.2	6.8
5 Treated	6.9	8.0	8.6
Control	5.0	5.2	5.4
Average:			
Treated	7.34	8.42	8.84
Control	5.70	6.46	6.80
Difference	1.64	1.96	2.04
Increase	28.8%	30.3%	30.0%

These experiments were not continued for more than three months because abundant production of stone cells in the bark made accurate measurements very difficult. Later measurements indicate, however, that the treated portion of the panel maintains its greater thickness for at least five months after treatment.

The increase in bark thickness due to the hormone application was

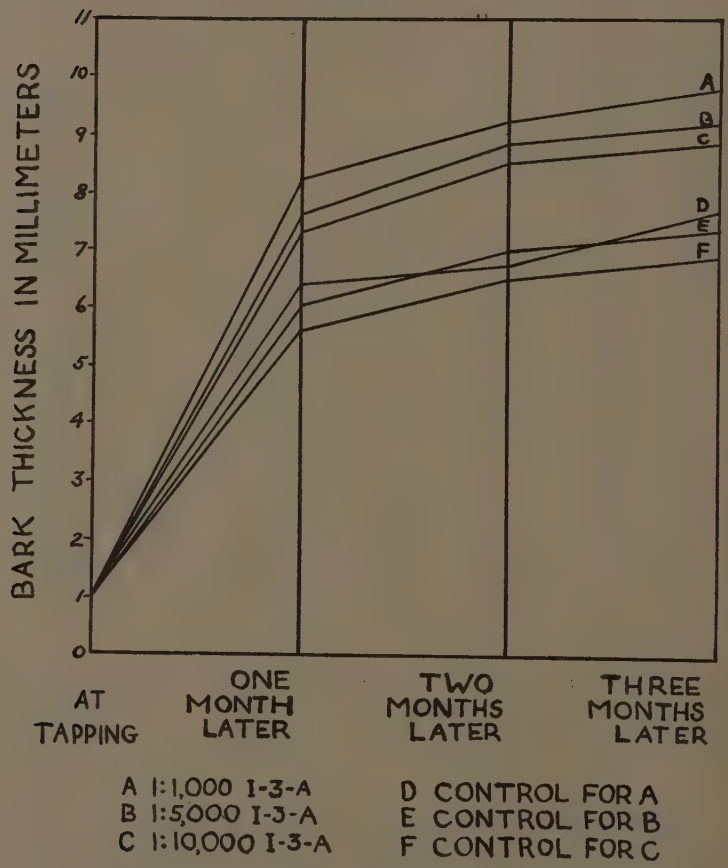

FIG. 1. Bark renewal in Avros 152 over a three-month period. A separate control is measured for each treatment (see text)

clearly visible as a swelling on the treated zone of the panel. A striking feature of this experiment was the greater quantity of latex exuding from the treated zone than from the untreated zone, possibly indicating a better developed latex system. As Bobilioff (3) has shown, formation of new latex vessels takes place very soon in renewing bark. However, definite proof that more vessels are formed in the treated zone must await histological examination.

The most rapid renewal of bark occurs in the first month after tapping; it tapers off in the second and third months. Figure 1 shows the relative rates of renewal over the three-month period. The 1 : 1,000 concentration exhibited the greatest effect, giving an increase over the control of 29.5 per cent in the first month, which rose to 35.3 per cent in the second month. In the third month the increase was only 26.5 per cent. The other concentrations proved to be somewhat less effective. The 1 : 5,000 solution was least effective.

EXPERIMENTS WITH LANOLIN AND INDOLE-BUTYRIC ACID

A second series of experiments was devised to determine how much of the increase noted in the previous one was due to hormone stimulation

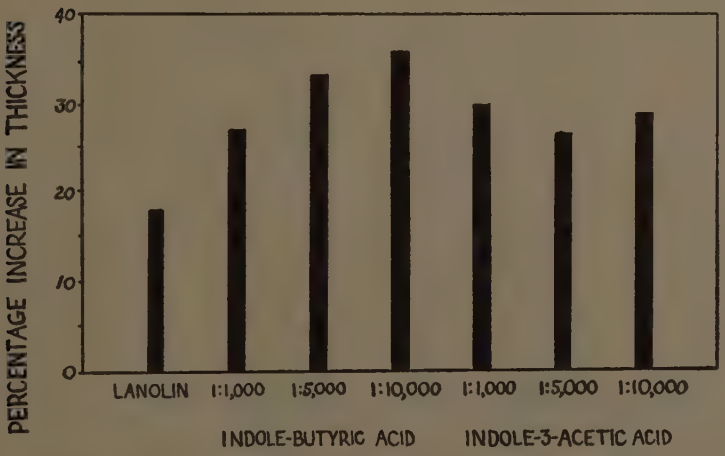

FIG. 2. Percentage increase of treated portion of panel over untreated (100 per cent) one month after treatment

and how much to the effect of the lanolin carrier in preventing desiccation. It was carried on for one month.

TABLE II

THE EFFECT OF LANOLIN AND INDOLE-BUTYRIC ACID ON BARK RENEWAL

In all these experiments the lower half of the panel was treated and the upper half served as a control. The figures give the bark thickness one month after treatment.

LINE D		LINE E	
Lanolin		1 : 5,000 indole-butyric	
Tree	*Mm.*	*Tree*	*Mm.*
1 Treated	6.7	1 Treated	7.2
Control	5.2	Control	5.3
2 Treated	8.2	2 Treated	8.1
Control	6.8	Control	6.4
3 Treated	5.8	3 Wind-damaged	
Control	4.8		
4 Treated	6.6	4 Treated	6.7
Control	6.0	Control	5.6
5 Treated	6.4	5 Treated	7.3
Control	6.0	Control	4.7
Average		Average	
Treated ...	6.74	Treated ...	7.33
Control ...	5.76	Control ...	5.50
Difference	0.98	Difference ...	1.83
Increase	17.0%	Increase	33.3%

LINE F		LINE G	
1 : 1,000 indole-butyric		1 : 10,000 indole-butyric	
Tree	*Mm.*	*Tree*	*Mm.*
1 Treated	8.1	1 Treated	7.4
Control	6.8	Control	4.7
2 Treated	7.3	2 Treated	9.1
Control	6.4	Control	7.3
3 Treated	7.6	3 Treated	7.1
Control	5.2	Control	5.4
4 Treated	8.6	4 Treated	7.4
Control	6.2	Control	5.4
5 Treated	6.7	5 Treated	7.7
Control	5.4	Control	5.8
Average		Average	
Treated ...	7.66	Treated ...	7.74
Control ...	6.00	Control ...	5.72
Difference ...	1.66	Difference ...	2.02
Increase	27.7%	Increase	35.3%

Pure lanolin and solutions of 1 : 1,000, 1 : 5,000, and 1 : 10,000 parts indole-butyric acid in lanolin were applied to the same clone, under the experimental conditions previously stated. The initial thickness of bark was one millimeter. The depth of renewed bark one month after treatment is shown in Table II.

Although lanolin alone increases the rate of renewal by 17.0 per cent, there is an additional stimulation due to the presence of the indole-butyric acid. This hormone is most effective at a concentration of 1 : 10,000, which gives an increase of 35.3 per cent or 2.02 millimeters one month after treatment. The relative percentage increment over the control is shown in Figure 2. The first-month increase in the previous experiment, which was due to the indole-3-acetic acid, is also shown for the sake of comparison.

DISCUSSION

Although the stimulating effect of indole-butyric acid becomes more marked as concentration decreases, this is not true of indole-3-acetic acid. Here the 1 : 1,000 concentration is most effective, at least in the early stages, and the 1 : 5,000 concentration shows the least effectiveness.

The effect of the lanolin is probably due to the fact that it protects the bark from desiccation, particularly during the dry season. Possibly this effect would not be so marked during the wet season.

SUMMARY

1. Experiments dealing with the effect of indole-3-acetic acid, indole-butyric acid, and lanolin on bark renewal are described.

2. The rate of renewal is most rapid in the first month after tapping.

3. Both the indole-3-acetic acid and the indole-butyric acid stimulated the rate of renewal.

4. Lanolin alone increased the renewal 17 per cent in the first month after application.

5. The treated zone of the panel exuded more latex when pierced by the bark gauge than did the untreated zone.

6. Indole-3-acetic acid is more effective at the highest concentrations used than at the lower concentrations, at least in the early stages.

7. Indole-butyric acid is most effective at the lowest concentrations used.

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LITERATURE CITED

1. Baptist, E. D. C. 1939. Plant Hormones. Journ. Rubber Res. Inst. Malaya, 9 : 17–39.

2. Beeley, F., and Baptist, E. D. C. 1939. Palm Oil Diluent for Tar Oil Fungicide and Its Effect on Bark Renewal of *Hevea*. Journ. Rubber Res. Inst. Malaya, 9 : 40–50.

3. Bobilioff, W. 1920. Some data on the Bark Renewal of *Hevea brasiliensis*. Arch. Rubbercultuur Ned.-Ind., 4 : 248.

4. Gandrup, J. 1921. On the Influence of Tar on *Hevea*-bark. Arch. Rubbercultuur Ned.-Ind., 5 : 561.

5. Haigh, J. C. 1928. Report on a Bark Examination of Peradeniya and Henerat-goda Rubber Trees. Ann. Royal Bot. Garden Peradeniya, 12 : 63–100

6. Keuchenius, P. E. 1918. Wound Curing and Healing with *Hevea* Trees. Arch. Rubbercultuur Ned.-Ind., 2 : 662–665.

7. La Rue, C. D. 1920. Bark Thickness in *Hevea brasiliensis*. Arch. Rubbercultuur Ned.-Ind., 4 : 45–64.

8. Sharp, C. C. T. 1937. The Treatment of the Pruned Surface of Large Stocks of Buddings of *Hevea brasiliensis*. Journ. Rubber Res. Inst. Malaya, 8 : 39–45.

9. Steinmann, A. 1921. Concerning the Influence of Coal Tar on the Renewing Bark of *Hevea brasiliensis*. Arch. Rubbercultuur Ned.-Ind., 5 : 504.

10. Vischer, W. 1921. Cork Formation and Bark Renewal in *Hevea brasiliensis*. Arch. Rubbercultuur Ned.-Ind., 5 : 493.

The trunk of a rubber tree (Hevea), showing tapping panel, spout, and glass cup full of latex. Area of panel between *A* and *B* treated with hormone. Upper side of panel, beyond *A*, untreated

FORESTRY

SOME RESUPINATE POLYPORES FROM THE REGION OF THE GREAT LAKES. XXI*

DOW V. BAXTER

IN 1922 the author began a survey of North American polypores. Since that time he has made field studies in every state of the Union, in Alaska, in every Canadian province as well as in the Yukon Territory, the Northwest Territories, and also in Labrador and Newfoundland (both of which have recently been annexed to Canada). In all reports of investigations and field expeditions (1) the title has been kept the same to preserve their unity and to facilitate references. It is the author's plan to incorporate in a volume on the resupinate polypores of North America the results of all these studies and to include additional data gathered since the publication of the various articles.

The present paper contains five reports on resupinate forms or pileate forms that have become resupinate.

The color terms given within quotation marks are those of R. Ridgway, *Color Standards and Color Nomenclature* (Washington, 1912).

Polyporus distortus (Schw.) Fr.

Abortiporus distortus (Schw.) Murr., Bull. Torr. Bot. Club, 31 : 422. 1904.
Boletus distortus Schw., Syn. Fung. Car., 71. 1818.
Polyporus abortivus Pk., Bot. Gaz., 6 : 274. 1881.

* Throughout the work on these monographs I have been indebted to many individuals and institutions for suggestions, help, and privileges extended to me. Grants from the Horace H. Rackham School of Graduate Studies, of the University of Michigan, have greatly facilitated both the field and the laboratory studies. My appreciation is expressed particularly to the men who have accompanied me on my numerous expeditions to Alaska, the Yukon Territory, and the Northwest Territories, and to Newfoundland and Labrador. Much credit is due them for aiding in the collection and care of specimens and for living, at times, under rather difficult circumstances. I am under obligation to Professor T. G. Halle of Naturhistoriska Riksmuséet in Stockholm, with whom I have had the pleasure of association. Thanks are due several American institutions and scholars also. To the authorities at the New York Botanical Garden, to the staff of the Division of Pathological and Mycological Collections of the United States Department of Agriculture, to Dr. John Stevenson for Plates V and VI, and to Professor H. H. Bartlett, of the University of Michigan, I am especially indebted.

Polyporus rufescens (Pers.) Fr. ex Lloyd, but held distinct by Murrill, Bull. Torr. Bot. Club, 31 : 422. 1904.

Fructification variable in growth habit, intertwined with roots and sticks or with central or lateral stem; stem often deeply extended and frequently tubercular to sclerotoid, extension which intertwines with decayed roots or wood, 8–12 cm. or more below the ground; pileus when developed 1–6 mm. thick, minutely but densely villose, "vinaceous buff"; context fibrous-tough, concolorous, duplex — the upper layer thin, the remaining radially fibrous; tubes 1–5 mm. long; mouths 0.5–3 to a mm.; spores[1] smooth, apiculate, ellipsoid to ovoid, 5–6 × 3.5–4 μ, chlamydospores 5–7 μ in diameter sometimes present, cystidia present or none, scarcely projecting beyond the hymenium; hyphae seldom branched, 4–8 μ in diameter.

Allied species. — The plant is described among the resupinate polypores since it may occur in a resupinate state about upright sticks. It suggests, for example, *Poria ambigua,* which at times may exhibit such a habit of growth. The pore mouths are similar in size, and the measurements of the spores for the two species overlap. There is no duplex context in *P. ambigua,* and the poria dries crisp and becomes so fragile that it is readily reduced to a powder between the fingers. *Pol. distortus* becomes harder upon drying.

As might be inferred from the similarity of names, resupinate forms of *Polyporus distortus,* which is especially variable in nature, may be mistaken for *Poria distorta* Murr. The tubes of *P. distorta* are given as shorter, being 1–2 to a mm. instead of 1–5 mm. long. Their mouths, too, are smaller. In *P. distorta* they are 4–5 to a mm., whereas in *Pol. distortus* the mouths are 1–3 to a mm.

Habitat. — *Acer Negundo, A. rubrum, A. saccharum, Ailanthus glandulosa, Betula nigra, B. papyrifera, Carya alba, C. illinoensis, Fraxinus sp., Juglans cinerea, J. nigra, Liriodendron Tulipifera, Magnolia grandifolia, Malus pumila, Plantanus occidentalis, Quercus alba, Q. palustris, Salix sp., Tilia americana, T. glabra.*

Distribution. — British Columbia, Ontario; Alabama, Arkansas, Connecticut, Delaware, District of Columbia, Florida, Illinois, Indiana, Iowa, Kentucky, Louisiana, Maryland, Massachusetts, Michigan, Missouri, Montana, New Jersey, New York, North Carolina, Ohio,

[1] Kauffman noted that in immature specimens or arrested-growth forms the pileus is a mass of pores filled with spores. Portions, at least, produce numerous conidia borne singly on slender sterigmata where present. The conidia are subglobose, and are about 10 μ in diameter.

Oregon, Pennsylvania, Tennessee, Texas, Virginia, Washington, Wisconsin.

Occurrence. — This plant may not always be wood-inhabiting, for it grows frequently about stumps and trunks of deciduous trees in low wet places. Kauffman found it around poison ivy roots and noted it in basswood and maple woods.

Cultures. — Isolated by Davidson, Campbell, Blaisdell (5). The fungus causes a positive reaction to tannic acid medium.

Trametes mollis (Sommf.) Fr., El. 71. 1828; Hymen. Eur. 585. 1874

Antrodia mollis (Sommf.) Karst. in Medd. Soc. pro Fauna et Flora Fenn., 5 : 40. 1879.

Daedalea mollis (Sommf.), Suppl. Fl. Lapp., p. 271. 1826.

Polyporus cervinus Pers., Myc. Eur., 2 : 87. 1825 (non *Trametes cervina* (Schw.) Bres., 1903.)

Polyporus mollis (Schw.) Kroh et Almqu., Svensk. Fl., 2nd edition, 5 : 274. 1932 (teste Pilát, 1936) (non *P. mollis* Fr., 1 : 360. 1821; nec Persoon, Myc. Eur., 2 : 62. 1825).

Trametes cervina (Pers.) Lloyd, non *Boletus cervinus* Schweinitz, Schriften Nat. Gesell., Leipzig, 1 : 96. 1822. (*P. cervinus* Fries, Epicr., 474. 1838; *Trametes cervinus* Bres., Ann. Myc., 1 : 81. 1903.)

Trametes stereoides (Fr.) sensu Bres. in Atti Ac. Agiati, III, 3 : 92. 1897. Shope in Ann. Mo. Bot. Gard., 18 : 367. 1921 (non *Polyporus stereoides* Fr., 1831).

Important specimen:

Trametes mollis Sommf., East Berne, Adirondack Mts. Leg. C. H. Peck (*Pol. cervinus* Pers.). Herb. N. Y. State Museum.

Fructification coriaceous, sessile, but more often effused-reflexed or resupinate, reflexed part conchate, 0–2 × 1–9 × 0.1–0.3 cm.; surface tomentose, zonate, brown, "Saccardo's umber" to "sepia"; margin thin, concolorous, becoming revolute; context 1 mm. or less in thickness, "cinnamon buff"; tubes up to 4 mm. long, concolorous with the context; mouths variable in shape, poroid, often becoming sinuous or daedaloid, 1–2 to a mm., "pinkish buff," "cinnamon buff" to "Saccardo's umber," spores hyaline, smooth, cylindric, 9–12 × 3.5–4.5 (Shope).

Allied species. — *Trametes mollis* (Sommf.) Fr. resembles *Trametes variiformis* closely. I suspect that, if specimens of *T. variiformis* were found in Europe, they might receive the name *T. mollis*, just as some coniferous collections have been so labeled in this country. The spores of the hardwood-inhabiting *T. mollis* (Sommf.) Fr. (7–10 × 2.5–3.5 μ) are about the same as those of the coniferous fungus *T.*

variiformis (6–8 × 2.5–3 μ). The tubes of *T. variiformis* are generally longer, but this character is only relative. The context of *T. variiformis* is duplex, the upper layer being concolorous with the surface, whereas in *T. mollis* the context is pale brown or clay color.

T. campestris Quél. is also closely allied, since it may resemble *T. mollis* in general appearance. The spores of *T. campestris,* however, are large (10–15 × 4.5–5.5 μ Pilát).

Romell (16) discusses a specimen of *Pol. stereoides* with pore mouths 4–5 to a mm. (Lapland). Its habit resembles that of a Stereum. The plant agrees with an authentic specimen from Femsjö in the herbarium of Fries. It is believed that Elias Fries either "suggested the name or at least approved it." Romell adds: "There is, however, another authentic specimen (with a label written by El. Fries), but this belongs to *Pol. cervinus* Pers." For the record Romell keeps this *Pol. cervinus* (*Daedalea mollis* Sommf., *Trametes mollis* Fr.) separate. According to him, it is "a species which is really closely allied, though in my opinion specifically distinct, *as it is much thicker and has pores more than twice as large and consequently a different habit,* and no intermediate connecting forms exist." *Pol. stereoides* is regarded as a northern plant and one never seen by Romell but in Lapland.

Difficulty has also been created by calling other and unrelated plants "mollis." Among these are certain more fragile polypores which are slightly red or which become reddish upon bruising or drying. *Pol. mollis,* for example, a soft, watery species when fresh, becomes rigid and red or pink when dry (17). It is an entirely different plant from *T. mollis.*

Irpex mollis should not be mistaken for *Trametes mollis. I. mollis* B. & C. is characterized by long (1 cm. or more) white teeth. The entire pileus is large and thick, remaining white or cream color in contrast to the browns exhibited by *T. mollis* (Sommf.) Fr.

Habitat. — *Acer circinatum, A. pennsylvanicum, A. saccharinum, A. saccharum, A. spicatum, Alnus rubra, Betula lutea, B. papyrifera, B. papyrifera kenaica, Fagus grandifolia, Fraxinus americana, Ostrya virginiana, Picea Engelmanii, Pinus Strobus, Populus balsamifera, Quercus alba, Q. Garryana, Q. phellos, Salix sp., Sambucus cœrulea, Ulmus americana.*

Distribution. — Alaska, Northwest Territories; British Columbia, Labrador, Nova Scotia, Ontario, Quebec; Arizona, California, Colorado,

Idaho, Illinois, Kentucky, Maine, Michigan, Montana, New Hampshire, New Jersey, New York, North Carolina, Ohio, Oregon, Pennsylvania, Rhode Island, Tennessee, Texas, Vermont, Washington, West Virginia.

Cultures. — Isolated by Davidson, Campbell, and Blaisdell (5), who report that the fungus gives a strong positive reaction to tannic acid medium.

Poria subserpens Murr., Lloydia, 10 : 245–246. 1947

(Plates I–III)

Trametes subserpens Murr., Mycologia, 12 : 107–108. 1920.

Trametes serpens Auct. Am., but Lloyd only pro parte (some determinations made by Lloyd are *Poria papyracea,* and not *Trametes subserpens*).

Elmeriana setulosa (P. Henn.) Bres., Philippines (?), teste Murr., Mycologia, 12 : 108. 1920.

Poria setulosa P. Henn. (?), teste Murr., Lloydia, 10 : 246. 1947.

Type and important specimen observed:

Trametes serpens Fr., "orig. ex." Fries. Femsjö, Sweden. Herb. of Bresadola, Stockholm.

Fructification white, pallid, or cream, corky, appearing in patches frequently about 4 × 3 cm., but becoming widely effused for as much as one or two meters, sometimes curled at margin but mostly resupinate, averaging 1 mm. in thickness; margin up to 1 mm. wide, "light buff"; tubes often arranged obliquely, 1–2 mm. long; hyphal pegs in tubes visible under hand lens (Overh.); mouths 1–2, mostly 2, to a mm., concolorous with the margin; dissepiments thick; spores 8–10 × 3.5–4.5 μ; hyphae 2–2.5 (3) μ in diameter.

Allied species. — *Poria subserpens* is one of the most distinctive of the resupinate polypores of the Gulf states. There has been some confusion about the name that should be applied, but no closely related species exist in continental North America. The name *P. straminea* Bres., designating a plant of the Philippines, has been variously interpreted, and some of the versions have been so broad as to include *P. subserpens*. Likewise, *P. setulosa* P. Henn. of the Philippines resembles *P. subserpens*.

Poria subserpens and *Trametes sepium* may be confused since both are coriaceous; they may resemble each other in color and in size of pore mouths. The dissepiments between the mouths are thick in both, and the two polypores are frequently entirely resupinate. The spores of *P. subserpens* are 8–10 × 3.5–4.5 μ, and those of *T. sepium* are 8–14 × 3.5 μ.

Poria subserpens is apt to be whiter than *Trametes sepium*. Furthermore, the relatively thin margin of *P. subserpens* is more likely to become upturned upon drying, with the tube layer remaining definitely resupinate. Drying affects *T. sepium* but little, and the margin in fresh plants becomes more effused-reflexed or even pileate in form, the pilei being often nodulose. A nodulose appearance is never evident in *P. subserpens*.

The two species, however, may be readily distinguished in culture by the fact that buff-colored pores are formed by *Trametes sepium* on both hardwood and coniferous blocks in one-year-old tests, whereas no pore formation takes place on either red gum or white pine in cultures of *Poria subserpens*.

Specimens of *Trametes serialis* may be mistaken for those of *Poria subserpens*. In nature *T. serialis* is chiefly a conifer-inhabiting fungus, whereas *P. subserpens* occurs on hardwoods.

Since resupinate specimens of *Fomes fraxinophilus* are corky, white or whitish, and possess a white to light-brown context, and since the mouths of the tubes average 2–3 to a mm., some difficulty may be encountered in separating it from *Trametes subserpens*. *F. fraxinophilus* grows on standing trunks, whereas *T. subserpens* is mostly, if not always, on logs. The spores of *F. fraxinophilus* are ellipsoid to ovoid, measure 6–8 × 5–6 μ (Lowe), and are not cylindric-ellipsoid, as in the species of Trametes discussed.

Some specimens of *Poria subserpens* might suggest those of *Trametes mollis* (Sommf.) Fr., which is never as white as most specimens (and all freshly growing plants) of *P. subserpens*. The margins of the two species are characteristic. At least, *T. mollis* is more often effused-reflexed, the surface of the pileus being tomentose, zonate, and brown. *P. subserpens* is usually more extensively effused over the substratum and often extends over the log for several feet. *P. subserpens* is mostly southern in its range and is common on Liquidambar and Lyssa. *T. mollis*, on the other hand, is mostly northern and is usually on species of Populus and Alnus. Both fungi, however, attack other woods.

Habitat. — *Acer floridanum, Carpinus sp., Carya sp., Celtis sp., Citrus aurantium, Ficus sp., Fraxinus americana, F. pennsylvanica lanceolata, Gleditsia aquatica, Liquidambar Styraciflua, Lysiloma bahamensis, Nyssa aquatica, N. biflora, Persea americana, Pinus echinata, Planera aquatica, Populus sp., Quercus laurifolia, Q. Shumardii, Q. virginiana, Salix nigra, Ulmus sp., Vitus sp.*

Distribution. — Alabama, Florida, Georgia, Illinois, Kentucky, Louisiana, Mississippi, North Carolina, New York, Ohio, Oklahoma, Pennsylvania, South Carolina, Tennessee, Texas, Virginia.

Occurrence. — *Poria subserpens* is one of the most common resupinate polypores of the states bordering the Gulf of Mexico. It is especially abundant on hardwood logs in tupelo-gum swamps. *Nyssa aquatica* is the usual substratum, but *Liquidambar Styraciflua* is frequently attacked.

Cultures. — Isolated from *Quercus Shumardii,* Fort White, Florida, and from *Nyssa biflora,* Stark, Florida. The snow-white mycelium from both isolates exhibits a concentric to radiating type of growth in malt-agar cultures in petri dishes. The two isolates are similar in their growth habits on wood, the mycelium covering both red-gum and white-pine test blocks in one-year-old flask cultures.

Poria subserpens grows faster in the dark than in the light. It also belongs to the rapid-growth group and in the category "high temperature" for optimum growth.[2] The white to "cartridge-buff" or "cream-buff" mycelium covers hardwood blocks (red gum), and eventually radiates in strands or fanlike growth over white-pine blocks, but often does not cover such wood as densely in one-year-old cultures. No pore formation has occurred.

Cultures exhibit a strong positive reaction to tannic acid medium.

Fomes subroseus (Weir) Humphrey and Siggers, Journ. Agric. Res., 47 : 999. 1933

(Plate IV)

Fomes carneus (Nees) Cooke, Grevillea, 14 : 21. 1885.
Fomes Palliseri Berkeley, Grevillea, 10 : 98. 1882. Saccardo, Syll. Fung., 6 : 204. 1888.
Fomes carnea granularis Pk., teste Pilát, 1936.
Fomes carnea subzonata Pk., teste Pilát, 1936.
Polyporus carneus Nees, Nova Acta Nat. Curiosorum, 13, t. 3. 1827.
Trametes subrosea Weir, Rhodora, 25 : 217. 1923.
Trametes arctica Berk., Herb., teste Pilát, 1936.
Trametes carnea (Nees) Pilát, Bull. Soc. Myc. Fr., 48 : 19. 1932.
Trametes carnea Cooke (sense of American students only).

Fructification annual or perennial, sessile, effused-reflexed, usually thin, coriaceous, 2–6 × 2–15 × 0.5–2 cm. (Shope), or resupinate; surface of pileus zonate or azonate, velvety tomentose, "pale drab gray," "wood brown" to blackish behind, becoming radially appressed, fibril-

[2] The terms used to represent various categories for growth are defined in Paper V (19 [1934] : 305–312) of this series.

lose, or glabrous; margin thin, sterile below, "light mouse gray," "light cinnamon drab"; context 1–5 mm. thick, corky, "fawn color," tubes concolorous, 0.5–4 mm. long each season; mouths "pale Quaker drab," "light gray vinaceous" to "russet vinaceous," concolorous, 3–5, mostly 4–5, to a mm.; basidia hyaline, 5–6 × 10–20 μ (Pilát); spores hyaline, smooth, cylindric, sometimes allantoid, 6–8 × 2–2.5 μ; no cystidia, hyphae seldom branched 3–5 μ (Shope) in diameter.

Forms.—*Trametes arctica,* listed here as a synonym, is regarded by Lloyd (10) as a form of *F. subroseus arctica* (Berk.) Lloyd with the surface silvery white and with appressed fibrils. The name *Pol. Palliseri,* also listed here as a synonym, was likewise applied to this plant by Berkeley.

Pilát keeps the name *Trametes carnea* and refers to the plant as *T. carnea* (Nees) Pilát. In view of the extensive use of the species name *subroseus* in pathological literature by Weir (24) and others (22, 25) and in view of the fact that Trametes is a doubtfully practical genus, it seems unwise to return to the use of the name *carnea.*

Allied species. — *Fomes subroseus* is most closely allied to *F. roseus.* The types of rot produced are indistinguishable. Although the pilei of *F. subroseus* are mostly thin, specimens are found occasionally that resemble the more ungulate types of *F. roseus.* The color of the mycelium of both species varies from white to pink, and the odor of cultures of the two is that of green apples. Nevertheless, the two polypores are distinct in their growth features and may be separated not only on the basis of their rates of development but also on optimum growth temperatures. (See page 51.)

Mounce and Macrae (12) found that both *Fomes subroseus* and *F. roseus* are heterothallic and bipolar with one exception, and that complete interfertility exists between haploid mycelia derived from different sources. The exception is of particular interest because it shows that two cultures of *F. subroseus* from widely separate sources possess one interfertility factor in common. These authors state that the two fungi may be differentiated on the basis of their spore characters. According to them, the failure to obtain clamp connections in any of the many pairings of a haploid mycelium of *F. subroseus* with a haploid mycelium of *F. roseus* only serves to emphasize that these two fungi are distinct.

Separation of *Fomes subroseus* from *F. roseus* on the basis of growth at certain temperatures has been made by Snell, Hutchinson,

and Newton (22). A test at 30° C. or 32° C. differentiates these two. *F. subroseus* grows over 32 mm. at 30° C., whereas *F. roseus* grows only about 24 mm. at this temperature. Evidence (22) is also presented to show that the two vary in moisture requirements, or in dryness tolerances with regard to fruiting. *F. roseus* is found in drier places, often on dry hewn timbers, but *F. subroseus* usually grows on logs covered with bark or on wood in situations that are decidedly moist (Pl. IV). *F. roseus* also occurs on worked timbers in the open and on decorticated bolts on top of log piles in well-ventilated places. *F. subrosea*, on the other hand, grows in low places and in parts of the pile protected from drying by grass and ferns.

Points of difference between the two species, *Fomes subroseus* and *F. roseus*, may be summarized as follows:

Fomes subroseus	*Fomes roseus*
1. Rate of growth in culture comparatively fast.	1. Rate of growth in culture comparatively slow.
2. Optimum growth temperature 28°–32° C. with most probable value at 31° C.	2. Optimum growth temperature 28° C.
3. Inhibiting temperature 38° C.	3. Inhibiting temperature 35° C. (22).
4. Requires moist sites with high humidities.	4. Requires relatively drier sites than *F. subroseus*.
5. Spores narrow, cylindrical, slightly curved, allantoid, 4–7 × 1–2 μ; smaller than those of *F. roseus*.	5. Spores elongate-ellipsoid, 5–7 × 2.5–3.5 μ; larger than those of *F. subroseus*.
6. Fruiting bodies generally longer and thinner than those of *F. roseus*.	6. Fruiting bodies generally thicker and less elongate than those of *F. subroseus*.
7. In general, no clamp connections form in pairings of a haploid mycelium of *F. subroseus* with one of *F. roseus*.	7. In general, no clamp connections form in pairings of a haploid mycelium of *F. roseus* with one of *F. subroseus*.

The European species, *Polyporus albocarneogilvidus* sensu Romell, might possibly suggest *F. subroseus* if only a small specimen without a part of the substratum were available for observation. *Pol. albocarneogilvidus* is Trametes-like. The mouths of the tubes are tinted slightly rose, and the fungus forms rather large coriaceous sheets, that is, they are often entirely resupinate.

Polyporus albocarneogilvidus is common around Stockholm on oak, and I have frequently observed it on dead limbs still attached to the trees. The habitat, the substratum, the color (which is not so distinct as in *F. subroseus*), and the larger mouths of this European polypore

should readily enable one to distinguish it from *T. subrosea.* I have never seen this European plant in America. The name *Poria micans,* as used by Bresadola with reference to some collections of *Pol. albocarneogilvidus,* is not acceptable.

Fomes subroseus Feei (*Trametes Feei* Fr.) (Pl. V, Fig. 1) is a tropical plant much like *Fomes subroseus* in size, shape, and color. The fibrillose surface, which is the only distinction between the two, is not always in evidence, and Lloyd (10) states that the two plants merge into each other. Snell, Hutchinson, and Newton (22) report as a result of their tests in comparing *F. roseus, T. subrosea,* and *T. Feei* that *T. Feei* in culture grows more slowly at all temperatures and is inhibited from growing at a lower temperature. It could be differentiated from the other two fungi if grown from 30° to 34° C. The type came from Brazil, and this is apparently the common plant of this group from Brazil. It is reported by Sharp (19) from Mexico and Guatemala.

Trametes Sagraeana, named by Montagne from Cuba, resembles *T. Feei,* though Lloyd (10) points out that most of Montagne's species under this name are *Trametes cupreo-rosea.*

Trametes Sagraeana has a smooth, soft, dull surface and smooth and very minute pores. It differs from *T. Feei,* according to Lloyd, in its more punky context and in having a surface soft to the touch, not hard and fibrillose. Lloyd finds *T. Sagraeana* rare in the West Indies (and believes the several collections from West Indies so referred at the New York Botanical Garden to be *T. cupreo-rosea*). He also states the abundant specimens so referred to by Murrill from Cuba are *T. Feei.*

Trametes cupreo-rosea (Pl. V, Fig. 2) is thin and rigid and is attached by a reduced base (6 × 9 × 0.5 cm.). The surface is striate-fibrillose, pale rosy color (buff pink). The context is thin and hard. The pores are round, medium to large, mostly about 0.5 mm. in diameter, with thin walls; tissue concolorous (10). Lloyd reports that a number of specimens from Brazil are at Kew, and there is a collection from Malay. He states that he has never seen a specimen from the West Indies. A collection of this fungus from Coconut Grove, Florida, is in the Farlow Herbarium, Harvard.

Trametes lilacino-gilva (Pl. VI), according to Lloyd, is in the genus Fomes, is the Australian analogue of *T. Feei* of American tropics, and has about the same color and surface. It differs in having notably larger pores and a more strongly fibrillose surface. The Aus-

tralian forms all have larger pores than the analogues in the American flora.

Trametes Eucalypti is, according to Lloyd, probably an Australian form of *T. lilacino-gilva* as to color and pores, but has an even pileus corresponding to that of *T. subrosea.*

Trametes roseola is an African species, close to *T. plebeia* in color, but differs in context. The surface is dirty rose, not zoned, and is slightly rugulose. The context is punky, dry, pale salmon ("light ochraceous salmon"). The pores are minute, rigid, 1–2 mm. long, and the spores are globose 4–5 (10).

Trametes plebeia in New Zealand, Indian, and Philippine plants has pinkish-cinnamon context, surface, and pore mouths. The surface of the fruiting body is smooth, dull, minutely velutinate, and soft to the touch; the context is hard and rigid (10). Lloyd (10) reports that Berkely referred the species to *T. plebeia* var. *cubensis* (*Polyporus supinus*) of American tropics, which is not a variety and has no analogy to it.

Habitat. — *Abies balsamea, A. grandis, Alnus rubra, Arbutus Menziesii, Betula occidentalis, B. papyrifera, occidentalis, Chamaecyparis thyoides, Juniperus virginiana, Larix laricina, L. occidentalis, Picea Engelmannii, P. excelsa, P. glauca, P. mariana, P. rubra, Pinus Banksiana, P. contorta, P. monticola, P. palustris, P. ponderosa, P. resinosa, P. rigida, P. strobiformis, P. Strobus, P. taeda, P. virginiana, Prunus amygdalus, P. pennsylvanica, P. persica, P. serotina, Pseudotsuga taxifolia, Quercus borealis maxima, Taxodium distichum, Thuja occidentalis, Tsuga canadensis, T. heterophylla.*

Distribution. — Northwest Territories; Alberta, British Columbia, Manitoba, New Brunswick, Newfoundland, Nova Scotia, Ontario, Quebec, Saskatchewan; Alabama, Arizona, California, Colorado, Connecticut, Delaware, District of Columbia, Florida, Georgia, Idaho, Illinois, Louisiana, Maine, Maryland, Massachusetts, Michigan, Minnesota, Montana, New Hampshire, New Jersey, New Mexico, New York, North Carolina, Oregon, Pennsylvania, Rhode Island, South Carolina, Tennessee, Vermont, Virginia, Washington, West Virginia, Wisconsin.

Occurrence. — *Fomes subroseus* is apparently unknown in Europe. It grows in most sections of the northern United States and Canada. It is not common in Newfoundland, but in general it is most frequent in areas north of the states that border the Gulf of Mexico.

The occurrence of *Fomes subroseus* in the field with reference to

that of its closely related ally, *F. roseus,* is significant. *F. subroseus* is most likely to grow on "unworked" timber in which moisture is more plentiful, as in moist log piles it is protected from drying out. *F. roseus,* on the other hand, occurs most frequently on "worked timbers," or on top of piles not so protected (22).

The occurrence of *F. subroseus* on Prunus in Oregon, where it causes much rot in stone-fruit trees, is added evidence of Davidson and Campbell's observation (4) that many normally coniferous species occur on Prunus among the hardwoods.

Cultures. — Isolated from tamarack, Whitmore Lake, Michigan. The fungus is characterized by a white to "shell pink" mycelial growth in culture. Some cultures remain almost white, whereas others may be slightly pink, or streaked with pink or "hazel" to "pecan brown" or "Rood's brown." Poroid areas form in wood-block cultures and appear often on malt-extract agar in petri-dish tests after two weeks. Basidiospores are produced. A few chlamydospores are formed, and clamp connections appear in abundance.

No reaction is exhibited on tannic acid medium.

The growth of *F. subroseus* may be considered moderately rapid and, in contrast with that of *F. roseus,* is so much faster at 31° C. that the two fungi may be distinguished on the basis of different growth rates (22).

Fomes subroseus belongs to the class of fungi that grow more rapidly in the light.

An isolate, reported by Humphrey and Siggers (8), exhibited an optimum temperature for growth at 28° C. The inhibiting temperature was 38° C. The fungus is placed, therefore, in their "intermediate-temperature" group (optimum between 24° and 32° C.).

The effect of moist heat and dry heat upon *Fomes subroseus* in three-quarter-inch wood-block cultures has been tested by Snell (21). This fungus was unable to withstand 131° F. (55° C.) for twelve hours at moist heat, whereas it took 221° F. (105° C.) twelve hours to kill the fungus with dry heat.

Reaction to chemicals. — Certain water-soluble extracts are toxic to *Fomes subroseus.* Water-soluble extract from western red cedar in concentrations of 4 per cent or over greatly retarded the growth of this fungus, according to tests made by Southam and Ehrlich (23).

Resistance of *Fomes subroseus* to sodium fluoride has been tested by Richards (15) and has been compared in its resistance to a num-

ber of other wood-destroying fungi. Beginning with the least resistant and ending with the most resistant, the list that follows indicates the relative position of *F. subroseus: Poria incrassata, Polystictus abietinus, Pol. versicolor, Fomes roseus, F. pinicola, F. subroseus, Lenzites saepiaria, Polyporus hirsutus, Fomes Pini* var. *Abietis, F. annosus* (possibly *Polyporus tulipiferus,* identity not certain), and *Lenzites trabea.*

Decay. — The rot caused by *Fomes subroseus* in conifers is identical with that brought about by *F. roseus.* In the incipient stages the wood turns somewhat darker than when sound, but the decayed parts are lighter in color than the brown to be observed in advanced stages of rot (7). Usually pink to red-brown patches appear in more or less elongated areas. These coalesce so as to form larger regions of decay. The wood shrinks on drying and breaks up into cubes.

In juniper there is at first a slight change from red to brown over a considerable area. As the discolorations deepen, fissures begin to appear. Pocket-like areas are formed about the fissures by the shrinkage of the wood on their periphery. Sometimes firm hard wood separates the punky portions and resists further decay. The pockets are most common near the base of the host, and here they often coalesce to form large irregular cavities filled with the brown charcoal-like wood. Overmature trees may be almost hollow at the base. Such specimens may be wind-thrown when the hollow becomes so great that the ring of sapwood cannot adequately support the tree.

In addition to causing rot in conifers, *Fomes subroseus* attacks peach and prune trees. The brown rot has been demonstrated to have been produced by the cytohydrolytic enzymes lignose, cellulose, and hemicellulose. Emulsin, also present in the mycelium, makes available the products from glucoside digestion in the wood (25).

Importance. — Comparatively few fungi which occur on structural woods attack both coniferous and hardwood trees as standing timber. *Fomes subroseus* is a conspicuous example of one fungus that does attack hardwoods and conifers, both living and fallen.

In the East it is commonly regarded as a scavenger fungus on dead coniferous wood, but if it occurs on pulpwood, the damage caused may be greater.

Frequently living junipers are attacked by *Fomes subroseus,* which produces a heartrot in them as well as in *Chamaecyparis thyoides.* A brown cubical rot appears in trees attacked (3).

Losses in prune orchards are large but difficult to evaluate. The damage to the diseased tree is not limited to the heartwood, since the sapwood is attacked. This results in destroying the water-conducting power by reducing the volume and by the stimulation of the growth of tyloses in the xylem vessels (25). Such disturbances give rise to "drouth effects" in the affected tops.

Zeller (25) conducted a survey of prune orchards to determine the extent of damage caused by *Fomes subroseus*. Of 1,261 prune trees in orchards having average horticultural care, 1,212 showed heartrot. Of these, 885 (or 73 per cent) were decayed by *Fomes subroseus*. The pruning cut in 619 (or approximately 70 per cent) of these decayed trees was two inches or more in diameter.

In some localities within the active range of the fungus vigorously growing older orchards do not seem to be affected by heartrot. The lack of heartrot in many orchards seems to be related to the system of pruning adopted by the grower.

Control and prevention in orchards. — In general, heartrot caused by *Fomes subroseus* is less prevalent in prune orchards where the system of pruning used eliminates operations on the main leader branches and trunk, particularly if trees are so trained in early years that the removal of large branches is unnecessary later. Wounds should be treated with an antiseptic dressing, such as Bordeaux paint, made by mixing linseed oil into a Bordeaux dust until a thick, smooth paint is formed (25).

Control and prevention in the forest. — Older trees are more susceptible than younger ones, so that cutting in relatively short rotations should be effective. For Tennessee juniper, Von Schrenk (18) estimated that, if cuttings are made between 65 and 70 years (when the trees average about 12 inches in diameter), substantial control of *Fomes subroseus* will result.

Though coniferous logs are usually removed from the woods soon after cutting, it is necessary at times to store them there. Care in woods storage becomes an especially important problem when large amounts of timber are felled by catastrophic storms (2). Complete submersion of the wood in fresh water before the decay fungus starts prevents rot, but damage from marine borers may result if the water is tidal or brackish. Deep submersion involves, however, high costs of recovery of the logs, and should therefore be avoided (2).

For storage of coniferous pulpwood in the forest, peeling delays

the action (9) of such a fungus as *Fomes subroseus,* and if the sticks are stacked on ends in open piles (and preferably off the ground), as is done frequently in Sweden, there will be less decay. Immediate peeling prevents bark beetles and wood borers from laying eggs in the wood, and this practice tends to reduce the incidence of rot. Such a procedure to delay fungus action may be especially justified financially if the wood is to be floated to the mill, a method used in some areas in North America. Debarking increases buoyancy of the wood.

In the North pulpwood is often cut in small lots and allowed to accumulate in the woods or at the point of shipment until a sufficient amount is obtained for selling in larger units. It may be stored for as much as two years before shipping, a practice that favors the development of *Fomes subroseus.* Upon arrival at the mill this wood is often handled like recently felled timber and thrown in with freshly cut sticks, so that additional losses from storage of all wood in the piles may be sustained. Such a practice should be avoided.

Control and prevention at the mill and in use. — In North America pulpwood is stored (*a*) in water, (*b*) on the ground in huge conical piles, and (*c*) in ricks. Water storage prevents decay if the wood is immersed. Log parts above the water level may decay if they remain in the same position for long periods. In the conical piling conditions are usually favorable for decay in the interior of the pile. Wood that has been water-transported from the forest to the mill can be protected from rot (and fire) by a system of overhead water sprays so regulated as to keep it soaked beyond the point favorable for fungus activity. It is impossible to soak dry wood in the piles to such an extent that decay will be prevented throughout the cones. Likewise, spraying of ricked wood, even if both ends of each log are exposed to the direct action of the water, is not generally satisfactory.

As a measure of rot prevention ricking pulpwood in piles raised from the ground on concrete or treated skids or piers is preferable to conical piling. The ricks should be separated by open spaces to allow ventilation. Yard sanitation is always necessary and, regardless of method of piling, the oldest wood in the lot must be used first.

In the South pulpwood deteriorates so rapidly that in general large quantities are not stored for more than four or six weeks. Often the fresh sticks are conveyed directly from barge, railroad car, or truck to the chipping machines.

The possibilities of reducing the activities of fungi in wood used in

buildings has been considered. *Fomes subroseus* belongs to the "intermediate temperature" or the "high-temperature" group that includes *Lenzites saepiaria, L. trabea,* and *Trametes serialis*. Hoxie (6) debated the possibility of using heat to retard the development of the comparatively "low temperature" or "dry-rot" fungi in buildings. It was concluded by Snell (21) from culture-block tests (see section entitled "Cultures") that, since a heating system could not supply a temperature much above 125° F., heating practices could not be effective in controlling *F. subroseus*. Too high a temperature for too long a period would be necessary to kill the fungus within the wood. Even for fruit-cannery buildings and other such structures of moist occupancy the heat required to kill such a species as *F. subroseus* or other species in this group could not be satisfactorily obtained. Dry heat, applied occasionally for the purpose of drying out the wood, is, nevertheless, to be recommended.

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LITERATURE CITED

1. Baxter, Dow V. 1927–1947. Some Resupinate Polypores from the Region of the Great Lakes, I–XX. Pap. Mich. Acad. Sci., Arts, and Letters, Vols. 6–34.
2. —— 1943. Pathology in Forest Practice, xi + 618. John Wiley & Sons, New York.
3. Boyce, J. S. 1948. Forest Pathology, xi + 550. McGraw-Hill Book Co., New York.
4. Davidson, Ross W., and Campbell, W. A. 1943. Decay in Merchantable Black Cherry on the Allegheny National Forest. Phytopathology, 33 : 965–985.
5. —— —— and Blaisdell, Dorothy J. 1938. Differentiation of Wood-decaying Fungi by Their Reactions on Gallic or Tannic Acid Medium. Journ. Agric. Res., 57 : 683–695.
6. Hoxie, F. J. 1930. Decay of Wood in Industrial Buildings. Fourth Edition, pp. 25–28, Inspection Dept., Associated Factory Mutual Fire Insurance Companies. Boston.
7. Hubert, E. E. 1931. An Outline of Forest Pathology, viii + 543. John Wiley & Sons, New York.
8. Humphrey, C. J., and Siggers, P. V. 1933. Temperature Relations of Wood-destroying Fungi. Journ. Agric. Res., 47 : 997–1008.
9. Kress, O., and Others. 1925. Control of Decay in Pulp and Pulpwood. U. S. Dept. Agric., Bull. 1298 : 1–80.
10. Lloyd, C. G. 1915. Synopsis of the Genus Fomes, pp. 211–287. Cincinnati.
11. Lowe, Josiah L. 1942. The Polyporaceae of New York. The New York State College of Forestry, 15 : 1–128.

12. Mounce, Irene, and Macrae, Ruth. 1937. The Behavior of Paired Monosporous Mycelia of *Fomes roseus* (Alb. and Schw.) Cooke and *Fomes subroseus* (Weir) Overh. Can. Journ. Res., 154–161.

13. Murrill, W. A. 1907. Polyporaceae. North American Flora, 9 : 1–72.

14. Pilát, Albert. 1936. Atlas des Champignons de l'Europe, Tome III. Polyporaceae I, 624 pp. Prague.

15. Richards, C. Audrey. 1924. The Comparative Resistance of 17 Species of Wood-destroying Fungi to Sodium Fluoride. Proc. Am. Wood Preservers' Assn., pp. 37–44.

16. Romell, L. 1911. Hymenomycetes of Lapland. Arkiv für Botanik, 11 : 1–35.

17. —— 1926. Remarks on Some Species of Polyporus. Svensk Botanisk Tidskrift, Bd. 20, H. 1 : 1–24.

18. Schrenk, H. von. 1900. Two Diseases of Red Cedar Caused by *Polyporus juniperinus* n. sp. and *Polyporus carneus* Nees. A Preliminary Report. U. S. Dept. Agric. Div. Veg. Phys. and Path., Bull. 21 : 1–22.

19. Sharp, Aaron J. 1948. Some Fungi Common to the Highlands of Mexico and Guatemala and Eastern United States. Mycologia, 40 : 499–502.

20. Shope, P. F. 1931. The Polyporaceae of Colorado. Ann. Mo. Bot. Gard., 18 : 287–456.

21. Snell, W. H. 1923. The Effect of Heat upon the Mycelium of Certain Structural Timber-destroying Fungi within Wood. Am. Journ. Bot., 10 : 399–411.

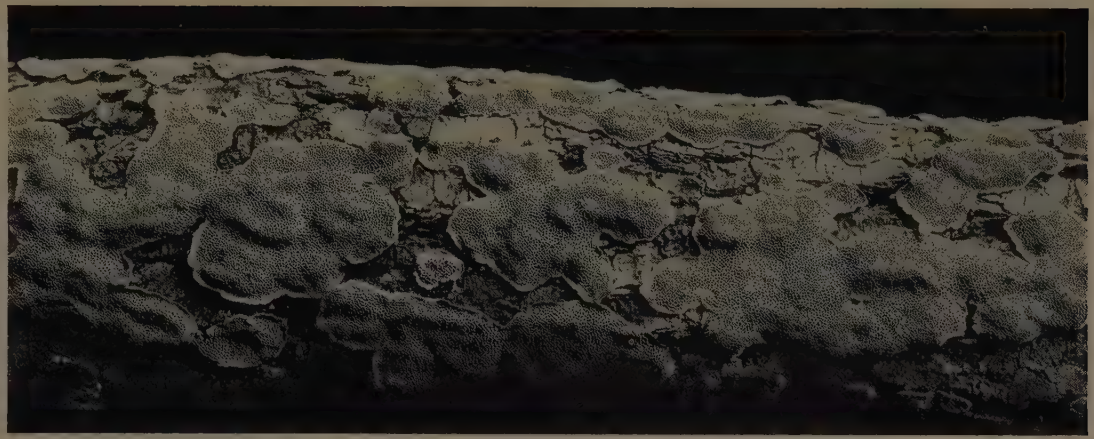
22. —— Hutchinson, W. G., and Newton, K. H. N. 1928. Temperature and Moisture Relations of *Fomes roseus* and *Trametes subrosea*. Mycologia, 20 : 276–291.

23. Southam, C. M., and Ehrlich, J. 1943. Decay Resistance and Physical Characteristics of Wood. Journ. For., 41 : 666–673.

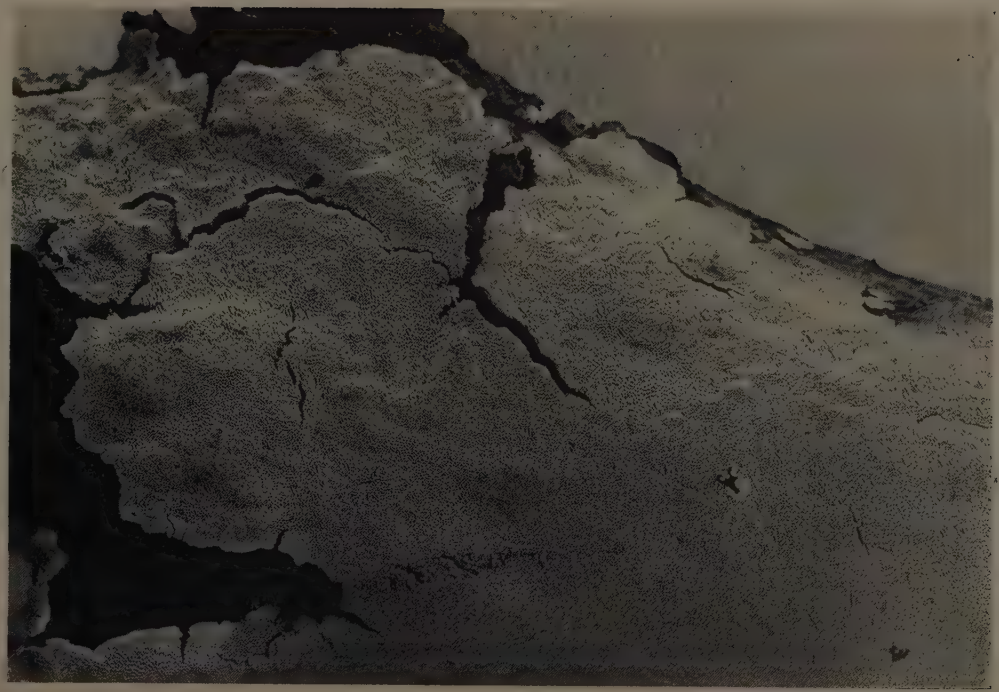
24. Weir, J. R. 1923. *Fomes roseus* (Alb. and Schw.) Cke. and *Trametes subrosea* Nom. Novum. Rhodora, 25 : 214–220.

25. Zeller, S. M. 1926. The Brown-Pocket Heart Rot of Stone-Fruit Trees Caused by *Trametes subrosea* Weir. Journ. Agric. Res., 33 : 687–693.

PLATES I–VI



Trametes subserpens on *Nyssa biflora,* Start, Florida



Poria subserpens on *Liquidambar Styraciflua*, Dismal Swamp, Virginia

Trametes subserpens, normally on southern hardwoods, but grows well on both white pine and red gum in culture. White pine blocks to left of center; red gum blocks to right. Top row. Isolate from *Nyssa biflora*. Middle row. Isolate from *Quercus Shumardii*. Botton row. Isolate from *Carpinus spp*.

Fomes subroseus on spruce, Winchendon, Massachusetts

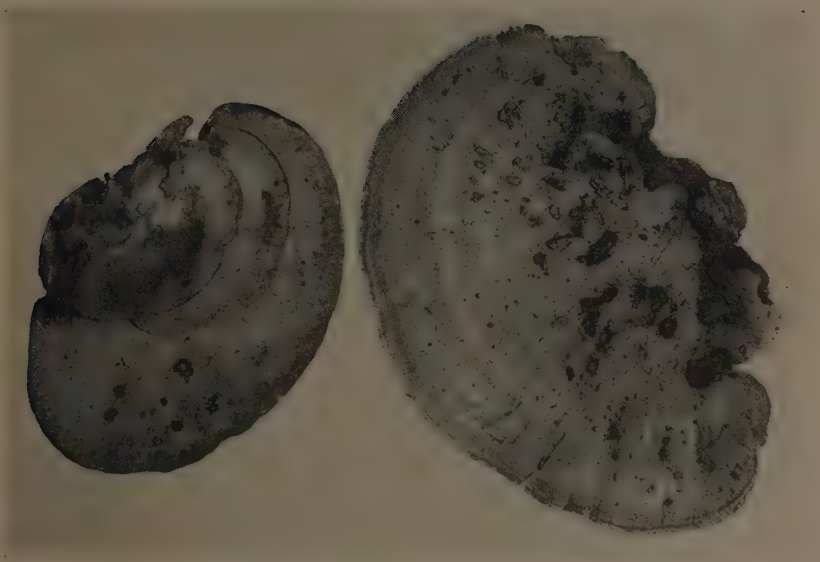
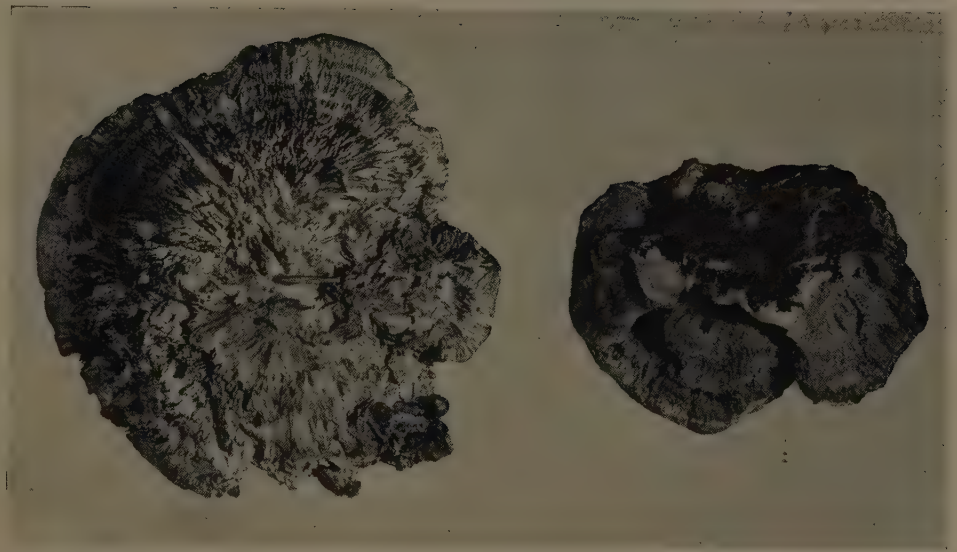


FIG. 1 FIG. 2

FIG. 1. *Trametes Feei,* Rio Grande du Sol, Brazil. 51788 Lloyd collection, Beltsville. Maryland. Morphologically this tropical plant appears to be similar to *Fomes subroseus,* but it grows more slowly at all temperatures. It can be further differentiated from *F. subroseus* and *F. roseus* if grown at temperatures from 30° to 34° C.

FIG. 2. *Trametes cupreo-rosea,* Bahia, Brazil. 17497 Lloyd collection, Beltsville, Maryland. This tropical plant has a thin pileus and is attached by a reduced base. A specimen so named from Florida is to be found in the Farlow Herbarium, Harvard



Trametes lilacino-gilva, East Caulfield, Australia. 45652 Lloyd collection, Beltsville, Maryland. Lloyd regarded this plant as the "Australian analogue" of *Trametes Feei* of the American tropics. This fungus needs to be compared with *T. cupreo-rosea*, *T. Feei*, and the other named species and forms in this group

RED PINE AND THE EUROPEAN PINE-SHOOT MOTH IN SOUTHERN MICHIGAN*

PAUL O. RUDOLF

RED PINE (*Pinus resinosa*) has been remarkably free from natural enemies. Its apparent immunity, combined with its ability to thrive on soils too light and dry for most farm crops, has made it a favored species for planting in southern Lower Michigan. About twenty years ago, however, a pest appeared in the state which now threatens to make the planting of red pine unsafe.[1] This enemy is the European pine-shoot moth (*Rhyacionia buoliana* Schiff.), the larvae of which kill or deform young pine shoots.

OCCURRENCE AND HABITS OF THE MOTH

Known for a long time in Europe as a serious pest of pines, the European pine-shoot moth was first found in the United States in 1914, on Long Island. Evidently it had been introduced on nursery stock from Europe (1, 2). It is now known to occur over the eastern United States as far south as Virginia and west to Michigan and in southern Ontario (3). Apparently it was brought into Michigan on nursery stock from Illinois. The larvae attack many pines, but in North America they prefer red, mugho (*Pinus mugo*), and Scotch (*P. sylvestris*) pines (4, 5).

The adult moths fly during early summer and lay eggs near the tips of twigs, either on the twig itself or on a needle sheath, usually on the upper part of the tree. Young larvae, which are hatched in about ten days, bore into the bases of the needles through the sheath, killing the needles affected. Later in the summer they penetrate the buds and cause a flow of pitch, which hardens into a whitish mass. They overwinter in the bud or under the mass of pitch on the bud. When the trees begin to

* Paper from the Lake States Forest Experiment Station, maintained by the U. S. Department of Agriculture, Forest Service, in coöperation with the University of Minnesota, at University Farm, St. Paul, Minnesota.

[1] During the past decade two other insects have also become serious enemies of red pine in the Lake States. These are the red-headed pine sawfly (*Neodiprion lecontei* Fitch) and the Saratoga spittlebug (*Aphrophora saratogensis* (Fitch)).

grow the next spring, the larvae bore into the developing shoots, which curl slightly and die. A little later in the spring, they become full grown, pupate in the burrows, and, in about three weeks, emerge as adults (3).

Trees up to thirty feet tall or taller are attacked, but ordinarily red-pine stands which are not infested until the trees are over ten feet tall do not become badly injured. Control is possible by cutting off and burning infested tips in late spring or by spraying with arsenicals (3) or DDT. As a rule, neither method is practicable in any but very small plantations.

The moth has a tendency to infest and reinfest the same trees for several years in succession. For this reason it can develop into a serious menace in a small area in from three to five years. For the same reason, however, it does not, by its own power, extend into new areas very rapidly.

AREAS OF INFESTATION IN MICHIGAN

While no detailed survey has been made, a dozen foresters and entomologists[2] familiar with conditions in Lower Michigan have made observations on the occurrence of the moth. Although these observations vary in intensity and accuracy, combined they present the best available overall picture of the extent of infestation in the Lower Peninsula.

There is some evidence that the moth was introduced into Michigan in the vicinity of Ann Arbor in the early 1920's. It was first found only on ornamental stock, but about ten to fifteen years ago it began to appear in forest plantations. From this point, and possibly from other places to which shipments of infested nursery stock were made, it has spread over the greater part of the southern half of the Lower Peninsula. It is now found westward to Lake Michigan and north throughout the Thumb, and as far as Bay, Midland, Mecosta, and Muskegon counties. The heaviest infestations are reported from Washtenaw, Wayne, Monroe, Livingston, and Shiawassee counties. Moderately heavy infestations have been noted in Saginaw, Jackson, Hillsdale, St. Joseph, Cass, and Berrien counties, and parts of the Thumb. In most of the other counties south of the Bay City–Muskegon line light infestations have been observed.

Generally speaking, ornamental and highway plantings are often

[2] The author appreciates information furnished him by Dr. Samuel A. Graham, of the University of Michigan; Dr. E. I. McDaniel and Lester E. Bell, of Michigan State College; Paul E. Slabaugh, of the Soil Conservation Service; and the following foresters of the Michigan Conservation Department: R. L. Olmstead, R. G. Auble, A. H. Boelter, I. H. McFarland, L. O. Miller, R. E. Pfeifer, A. J. Phillips, and Paul Schroeder.

badly injured, especially where mugho pine has been included. Indications are that red pine is injured on poor sites more than on good ones. Within plantations insect attacks usually are spotty. In some places 75 per cent or more of the trees are damaged, whereas a short distance away all or most of the trees may be undamaged. Infestations usually do not appear until trees have become two to four feet tall. On small trees injury occurs largely in the tops; taller trees often have much of the infestation in side branches. Plantations bordering hardwood stands appear to be relatively free of the moth.

POSSIBLE AREAS OF INFESTATION

The natural range of red pine extended just into the northern part of the southern half of Lower Michigan, but the original stands were removed long ago. Over the years, however, numerous small plantations have been established in this area. As a rough estimate, perhaps 10,000 acres have been planted to it in the thirty-seven counties of the southern half. In this area there are about 66,000 acres of forest land in need of planting on which red pine would be the preferred species. There is also a considerable area suited for this species, which is now in cropland or pasture that should be planted to trees.

CONTROL OF THE MOTH

The following methods of control have been recommended by entomologists: (1) in the late spring cut off and destroy infested twig tips; (2) when the adults emerge apply a spray, made up of (*a*) three pounds of lead arsenate and one quart of fish oil in 100 gallons of water, and repeat it ten days later, or (*b*) three pounds 50-per cent wettable DDT, plus three pounds black leaf 155 in 100 gallons of water, or (*c*) miscible oil and DDT.

One landowner in the Thumb had some twenty acres of plantations which were hard hit by the moth. By picking off infested tips and spraying the trees with 2 per cent DDT powder, he kept damage to a minimum, but this method requires careful watching and a good deal of labor.

It is necessary to spray only the outer edges of individual trees or the borders of large plantations. Yet this method of control is too expensive for general use. Large blocks of plantations probably could be sprayed economically from airplanes or helicopters, but so far there are few such areas in southern Michigan.

Natural control may be more economical than spraying. Already heavily parasitized specimens of pine-shoot moth larvae have been found in Monroe County, and artificial release of parasites along with their natural spread might be quite effective. But the most effective natural control probably is cold winter weather, since the larvae die at temperatures of about −18° F.

THE CLIMATIC ZONE OF NATURAL CONTROL

To learn where cold weather capable of curtailing the spread of the moth occurs, an analysis of the weather records of the last two decades was made for seventy-five places in Lower Michigan, and the number of years in which there were temperatures of −18° F. or lower were plotted on a map (Fig. 1).[3] Areas with similar numbers were grouped into five zones, which are listed below, together with the reasons for grouping them:

Zone 1. — Temperatures of −18° F. or colder occurred once or not at all. In this zone the insect can build up severe infestations and spread with little hindrance.

Zone 2. — Lethal temperatures occur one to three times, or, on the average, about every ten years. Severe infestations can be built up, but the spread of the insects will be more retarded than in Zone 1.

Zone 3. — Lethal temperatures come three to five times, or about every five years. Local infestations can become heavy, but there will not be much opportunity for their spread.

Zone 4. — About every three years, or five to ten times, there will be killing temperatures. Some local infestations may become relatively heavy, but there will be little opportunity for extensive serious attacks or for spread to new localities.

Zone 5. — Killing temperatures occur ten or more times, or every year or two. There is little opportunity for the insect even to establish itself.

If this reasoning is correct, severe damage from moths can be expected in red pine planted in southeastern Michigan within a narrow strip along the St. Clair River and the southern tip of Lake Huron, and within a nar-

[3] Some weaknesses in this procedure are recognized: (1) the duration of the low temperatures is not known; (2) for most of the southern stations the lethal temperature occurred only once in the year recorded, but for others such temperatures occurred two or more times in the year; (3) temperatures at the point where larvae lived may have been either higher or lower than those recorded at weather stations. However, these data are the best available and, despite their drawbacks, are useful.

row strip along Lake Michigan from about Ludington to the Indiana border (Zone 1 in Figure 1). Throughout most of the rest of the southern

ZONE SYMBOLS AND NO. OF OCCURRENCES OF -18°F. OR COLDER IN 20 YEARS

1 0-2

2 2-3

3 3-5

4 5-10

5 10-15 & 15-19

FIGURE 1

half of Lower Michigan (Zone 2) red pine may eventually become heavily damaged. Individual infestations may become severe, but their spread is apt to be highly restricted in portions of Lapeer and Tuscola counties; portions of Clinton, Ionia, Kent, Eaton, Barry, Allegan, Kalamazoo, and Van Buren counties; and in a belt including parts of Arenac, Bay, Midland, Isabella, Mecosta, Gratiot, Montcalm, and Newaygo counties, and extending north to the Lake Michigan shore and up to Charlevoix, and along the Lake Huron shore in Alcona, Alpena, and Presque Isle counties (Zone 3). Throughout the rest of the northern half of the Lower Peninsula the moth either is not likely to establish itself or is not apt to cause serious damage (Zones 4 and 5). For many purposes Zones 1 and 2 could well be combined.

CONTROL MEASURES PROPOSED

In view of the facts now known certain recommendations can be made to minimize the damage from the European pine-shoot moth in Lower Michigan. Some of them are as follows:

1. New red-pine plantations should not be established adjacent to plantings infested with the moth.
2. Present infestations should be controlled wherever additional plantings are contemplated. Special efforts should be made in the western part of the state, where the moth does not yet appear abundant and where rather extensive red-pine plantations already exist.
3. In the eastern tongue of Zone 1, where the infestation is most severe, suitable races of the more resistant ponderosa and Austrian pines should be sought as substitutes for red pine.
4. Red pine should not be planted on poor sites.
5. The use of mugho pine should be discouraged wherever red- or Scotch-pine plantations are to be established.
6. Unless constant vigilance is exercised and controls are applied at the first sign of infestation, red pine should not be planted in the southern half of Lower Michigan, except in Zone 3. Even here some damage is likely to occur if no artificial control is used.
7. From a long-range point of view red pine trees apparently resistant to damage from the moth should be utilized as a source of seed or breeding material for developing resistant planting stock.

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LITERATURE

1. Friend, Roger B. 1931. The European Pine Shoot Moth in Red Pine Plantations. Journ. For., 29 (4) : 551–556.

2. —— and West, Allen S., Jr. 1933. The European Pine Shoot Moth (*Rhyacionia buoliana* Schiff.) with Special Reference to Its Occurence in the Eli Whitney Forest. Yale Univ. School of For. Bull. No. 37, 65 pp., illus.

3. —— 1936. The European Pine Shoot Moth (*Rhyacionia buoliana* Schiff.). Tree Pest Leaflet No. 3, 4 pp., illus. Mass. For. and Park Assn.

4. McDaniel, E. I. 1930. European Pine Shoot Moth Found in Michigan. Mich. Agric. Exper. Sta. Quart. Bull. 13 (2) : 69–71.

5. —— 1938. Some Chewing Insects Infesting Michigan Evergreens. European Pine Shoot Moth. Mich. Agric. Exper. Sta. Circ. Bull. 141, pp. 4–7, illus.